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GROWTH HORMONE THERAPY IN CHRONIC KIDNEY DISEASE

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INTRODUCTION

In the last few years, there has been a shift in emphasis on the medical management of children with chronic kidney disease (CKD) from strategic attempts to preserve renal survival to optimizing global biological potential, and thereby maximizing quality of life. Early diagnosis and prompt treatment have become the cornerstones of modern care. Thus, in addition to measures like anemia control and improved nutritional intake, there is increasing use of recombinant human growth hormone (rhGH).

Although the FDA-approved indication for use of rhGH in CKD is growth failure, there are other clinically significant metabolic effects of the hormone. In this review, we shall highlight the potential benefits of rhGH therapy in CKD, including its positive impact on cellular growth and metabolism, immune regulation, and energy homeostasis. The roles of rhGH in modulation of psychosocial function, sleep physiology, and bone metabolism in children with CKD will also be discussed.

GROWTH FAILURE

More than 50% of adults with childhood-onset CKD attain final heights that are below the third percentile.[1] The burden of growth retardation in patients with renal disease is enormous, resulting not only in physical handicaps but also the potential for psychological and social distress.

CKD, whether caused by congenital anomalies, chronic infection, immune disorders, or connective tissue diseases, may be associated with nutritional deficiency and

From The Editor's Desk

This issue contains 9 printed and 8 e-abstracts of important papers in the field with editorial comments. Thus, we have doubled the content of *GGH* with the introduction of e-abstracts. This feature also allowed the publication of longer abstracts, data, and comments. Please take advantage of this added feature. I welcome your comments regarding the on-line and print aspects of the journal. Your feedback is important as we continue to grow and strive to serve your needs. The lead article by Drs. Bamgbola and Kaskel on Growth Hormone Therapy in Chronic Kidney Disease is a very comprehensive review of the pathophysiology of the disease as it pertains to growth hormone. It includes provocative ideas about possible future direction for treatment with growth hormone and insulin-like growth factor. I am sure you will enjoy it and save it as a reference source.

The expanded journal and all of its content is carefully prepared by the editorial board and all the lead articles are reviewed to comply with the high scientific standards of a peer review journal. *GGH* is also in compliance with the code of conduct for medical publishers on the internet (Health on the Net Foundation www.hon.ch).

Respectfully,
Fima Lifshitz, MD

Highlights In This Issue

E-Abstracts (Abstracts On-line)

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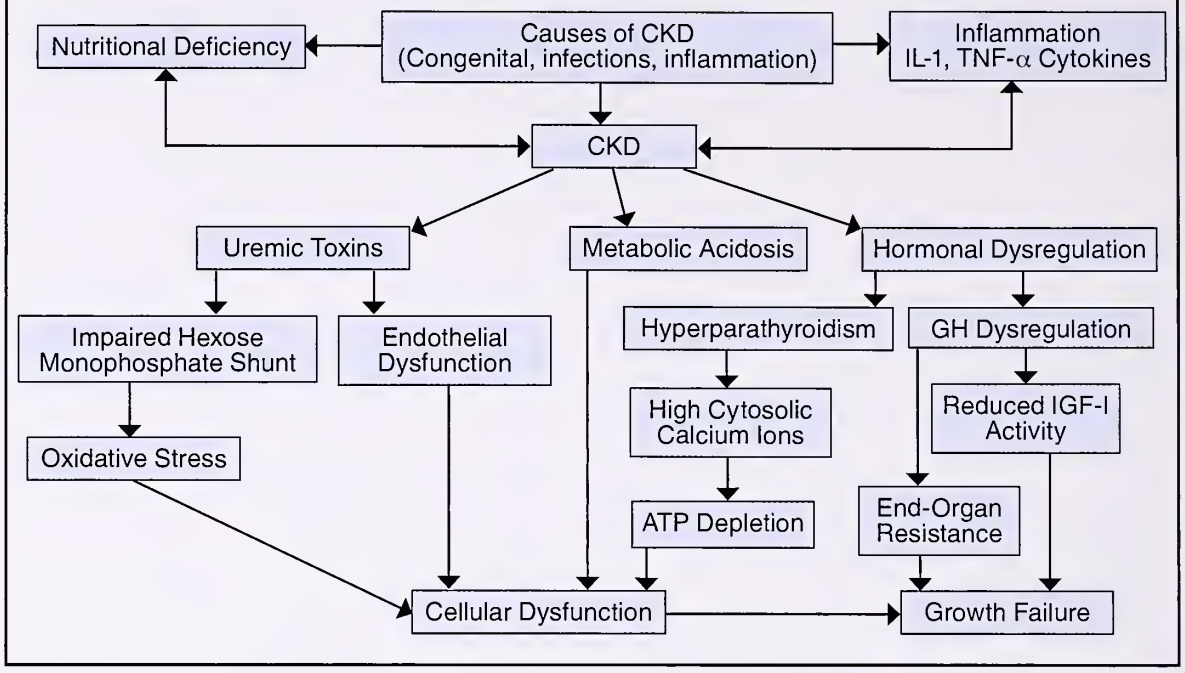
growth retardation (Figure 1). Conversely, consequences of renal disease such as metabolic acidosis, endocrinopathy, chronic anemia, persistent micro-inflammation, recurrent infection, and cardiac dysfunction may also result in growth failure. Inadequate dietary intake (often less than 80% of RDA) and defective protein metabolism are common features of CKD. However, increased food intake does not necessarily translate into a healthy nutritional outcome, and it often leads to greater adiposity rather than musculo-skeletal growth.

Figure 1 ABNORMAL CELLULAR METABOLISM AND GROWTH IN CKD



Furthermore, metabolic acidosis, which is a common outcome of CKD, accelerates protein degradation by activation of the ubiquitin-proteasome pathway, stimulation of branched-chain keto-acid-dehydrogenase, and promotion of end-organ resistance to anabolic effects of GH.[2] In addition, steroid therapy, often used as an anti-inflammatory agent in some kidney diseases, or for immune suppression following renal transplantation, may not only impair GH release but also increase end-organ resistance. In this regard, there is a positive correlation between the cumulative dose of steroids and adult-height deficit in pediatric allograft recipients. Treatment with steroids may inhibit GH synthesis by stimulation of (hypothalamic) somatostatin production. Consequently, by acting on multiple receptor-sites of the pituitary gland, GH-releasing peptide-2 (a GH secretagogue) has the therapeutic potential of bypassing the inhibitory effect of somatostatin.[3] Similarly, the use of rhGH alone or in combination with insulin-like growth factor (IGF)-I promotes musculo-skeletal growth, essentially by attenuating the inhibitory effect of steroids on protein synthesis.[4]

Whereas somatic growth at an early age is predominantly determined by factors such as birth size and adequate nutritional status, functional availability of GH is essential during childhood, and gonadotropin is a necessary adjunct for post-pubertal maturation.[1] Consequently, provisions of an optimal metabolic and nutritional milieu are often sufficient for growth in children with CKD who are less than 2 years of age, while use of rhGH is commonly required in older children.

GH/IGF AXIS

Although the pulsatile release of GH is blunted in uremia, the total amount of GH secretion from the pituitary gland is often increased.[5] IGF-I and -II are derived from both hepatic cells and local tissues (of target organs) in response to a primary activation of the GH receptor (GHR).[6,7] Despite the higher plasma level of circulating GH,[8] there is less synthesis of IGF-I due to end-organ resistance.[9]

Factors that contribute to GH tissue resistance in CKD include hyperparathyroidism, metabolic acidosis, and pro-inflammatory cytokines.[9-12] The mechanism of the end-organ resistance is inhibition of calcium-mediated intracellular signaling and impaired transcription of GHR-mRNA. Thus, GH activation of growth plates in uremic animals results in reduced local synthesis of IGF-I, impaired chondrocyte replication, and therefore retarded skeletal growth.[13]

The physiologic functions of GH are mediated by 2 different but complementary mechanisms: GH directly activates target organs while its indirect effects are mediated through IGF-I.[7] While GH increases the hepatic production rate of glucose and glycerol (an index of lipolysis), IGF-I acts in concert with insulin to increase peripheral glucose uptake and to reduce protein breakdown.[14]

IGF-I is a small, single-chain peptide belonging to the same family of genes as IGF-II and pro-insulin,[15] and its free bioactive form accounts for 1% of total plasma concentration.[7,16] IGF-I has a very short half-life (20 minutes), rapidly losing its metabolic function in the absence of a carrier binding-protein (IGFBP).[6,7] The most abundant of the 6 IGF-binding proteins (IGFBP-1 to -6) is IGFBP-3; it binds to circulating IGF-I and acid labile-sub-unit (ALS) as a 150 kDa ternary complex, thereby protecting it from premature degradation.[7,16]

IGF-I receptors are heterotetramers comprised of 2 alpha and 2 beta sub-units attached by disulfide bridges. IGF-I ligand binds to the extracellular alpha sub-unit which in turn induces the transmembrane beta unit,

resulting in an autoactivation of tyrosine kinase and phosphorylation of an intracellular tyrosine residue.[15] Interaction between insulin receptor substrates (IRS-1 and -2) and the receptor-tyrosine residue evokes a signal transduction thereby activating the downstream MAP-3 kinase (and protein kinase-B) pathways.[15] The 2 pathways mediate protein synthesis, cellular growth, cell motility, and inhibition of apoptosis.

IGFBP-3, by sharing a similar molecular structure, competitively inhibits IGF-I receptors.[15] However, the receptor molecules have stronger affinity for the IGF-I ligand. Consequently, there is a regulated but slow release of the plasma IGF-I from its carrier proteins at the designated target tissue. In uremic plasma, IGFBP-3 peptides are more rapidly degraded into smaller fragments. The smaller molecules of IGFBP-3 have less avidity for IGF-I and are often poorly excreted by the diseased kidneys. The reduced renal clearance of the relatively inefficient IGFBP-3 fragments and retention of inhibitory binding proteins, including IGFBP-1, -2, -4, and -6, substantially reduce the bioavailability of IGF-I.[16,17]

Future Directions for GH/IGF-I Treatment

Despite end-organ resistance to GH in uremia, exogenous administration of rhGH accelerates skeletal growth by increasing the molar ratio of IGF-I to IGFBP-3. However, CKD patients often require dose levels of rhGH 2 to 3 times higher than doses administered to GH-deficient subjects.[7] In addition, combined therapy with rhGH and rhIGF-I results in a greater than additive effect, or synergistic interaction, in CKD patients.[6]

Given the prevalent organ resistance to GH in CKD, therapeutic approaches that increase functional availability of IGF-I may be more effective than the simple administration of rhGH as is currently practiced.[6,7] These measures may include the use of exogenous IGFBP-3 to replace the inhibitory smaller fragments and IGF-I analogs to displace endogenous IGF-I from its binding proteins.[6,7] While the binding protein may prolong the half-life of IGF-I, IGF-I analogs may increase the effective concentration of the bioactive free IGF-I. Therapeutic administration of combined IGF-I and IGFBP-3 complexes have been successfully used to enhance positive nitrogen balance in burn patients.[6]

Furthermore, synthetic GH-releasing peptide (GHRP) and its endogenous equivalent, ghrelin, may be available for oral administration in the near future.[7] These GH secretagogues are more potent than the conventional GH releasing hormone (GHRH) in stimulating a pulsatile release of GH. They act on specific receptors of the anterior pituitary gland, thereby restoring its normal physiologic characteristics. These include capacity for feedback regulation and a greater than 6-fold increase in IGF-I synthesis.[6] This therapeutic approach has been introduced into clinical practice with the combined use of GHRP and thyroid-releasing hormone to reactivate pulsatile pituitary secretion of GH and thyroid-stimulating hormone, thereby preventing protein catabolism and muscle wasting in protracted critical illness.[18]

Delayed Puberty, Hypogonadism, and rhGH

There is a complex interaction among GH, IGF-I, and sex steroids in maximizing growth potential and body composition and in promoting sexual and reproductive capacities in human subjects.[19] Although the mechanism is unknown, the increase in pituitary GH synthesis during mid-puberty in boys is preceded by an increase in plasma testosterone. Similarly, the GH/IGF-I axis is activated by small increases in plasma estrogen in girls at the onset of puberty. GH and IGF-I influence reproductive function directly by modulation of gametogenesis and indirectly by enhancing steroidogenesis. Achievement of critical body weight is associated with pubertal onset, suggesting that somatic effects of rhGH treatment may play a role in the attainment of spontaneous puberty.[20,21]

The common findings of hypogonadism and delayed puberty in CKD are characterized by a loss of the normal pulsatile hypothalamic release of gonadotropin-releasing hormone (GnRH).[22] Puberty may be delayed for up to 2 years, while peak height velocity is often less than 50% of normal in CKD patients. There is a low expression of GHR in a GHR gene knockout-mouse model, similar to the findings in human CKD subjects. These mice have delayed maturation of seminal vesicles, spermatids, and testes, with a poor testicular response to leutinizing hormone, supporting a role for rhGH in induction of pubertal maturation.[23] The use of rhGH/IGF-I administered with GnRH analog (experimental hypogonadism) in men has been shown to preserve protein synthesis and lipid oxidation compared with controls, indicating an independent effect of the combined regimen in the maintenance of fat-free mass.[24] Similarly, combined therapy with rhGH and testosterone synergistically promotes muscle IGF-I gene expression, whole body protein anabolism, bone turnover, physical performance, and sexual function.[25,26]

METABOLIC CHANGES AND rhGH THERAPY

Insulin and Glucose Metabolism

Insulin and glucose metabolism in CKD (Figure 2) is characterized by reduced activity of glycolytic enzymes with a consequent decrease in glycolysis, glycogen synthesis, and storage. In uremic rats, there is 25% to 45% reduction in hepatic gluconeogenesis and glucose formation rate from fructose and pyruvates.[9] Similarly, due to a defective intracellular (post-receptor) signaling there is impairment of hepatic insulin metabolism in uremic rats. In addition, although pancreatic insulin secretion is reduced, its renal degradation is substantially compromised in CKD. The resultant hyperinsulinemia stimulates plasminogen activator inhibitor,

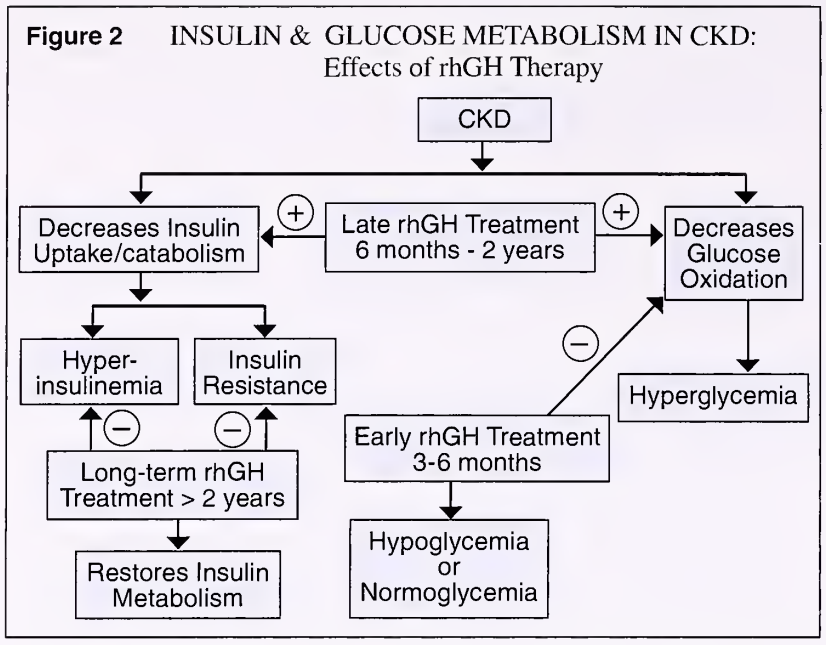


Figure 2 INSULIN & GLUCOSE METABOLISM IN CKD: Effects of rhGH Therapy

reduces fibrinolysis and, therefore, promotes vascular thrombus formation.

rhGH Therapy and Glucose Metabolism

In the early phase of rhGH therapy, insulin-like effects (including hypoglycemia and protein synthesis) predominate and serve to overcome the uremic-induced insulin resistance (Figure 2). This effect is due to a cross-affinity of IGF-I with insulin receptors leading to an increased glucose uptake and cellular oxidation.[27] On the other hand, with long-term rhGH administration, there is impairment of insulin-mediated glucose uptake, increased lipid oxidation, and formation of insulin-resistant (glycolytic type II) muscle fibers.[28] Consequently, hyperglycemia ensues with an increase in glycosylated hemoglobin. In general, restoration of normal glucose tolerance has been shown to occur within 2 years of starting rhGH therapy.[29,30] These paradoxical effects of rhGH may result from functional and structural diversities of its fragments. For example, GH fragment 1-15 is endowed with insulin-like effects, whereas GH fragment 177-191 possesses anti-insulin properties, and the 20K-GH variant promotes cellular growth.[31]

Protein Metabolism in CKD

Although hepatic synthesis of total serum protein is often preserved in CKD subjects, production of specific proteins such as IGF-I and apolipoprotein A1 are commonly reduced.[9] Similarly, there is a 30% to 40% reduction in enzymatic activity of the urea cycle, with a down-regulation of ureagenesis and accumulation of nitrogenous substances, including middle molecule toxins (poorly dialyzed, larger-sized uremic molecules) such as advanced glycation end products, and ß2-microglobulin.[9]

As previously stated, metabolic acidosis and uremic-induced inflammation cause protein degradation by activation of ubiquitin-proteasome pathway, induction of branched-chain ketoacid dehydrogenase, and promotion of end-organ resistance to insulin and GH/IGF-I (Figure 3). The physiologic impact of activated uncoupling proteins (UCP polymorphism) on mitochondrial oxidative phosphorylation is substantial and may account for up to 20% of basal energy expenditure.[32] Tumor-necrosis factor (TNF)-α cytokine, often elevated in CKD, promotes negative nitrogen balance by up-regulating UCP-2 and -3 genes in skeletal muscles of experimental rats.[33]

rhGH Therapy on Protein Metabolism

Treatment with rhGH increases protein synthesis, not only by stimulating uptake of amino acid, but also by promoting intracellular peptide assembly.[34] Protein degradation is prevented by inhibition of lysosomal and ATP-ubiquitin-proteasome pathways. Thus, the net effect of rhGH therapy in CKD is an efficient use of dietary branched-chain amino acids with improved skeletal muscle performance.[35,36] Consequently, administration of rhGH therapy after long-term mechanical ventilation has been shown to result in improved respiratory muscular strength, reduction in ventilator settings, and successful extubation in post-surgical patients.[37] Similarly, combined use of GH/IGF-I as an adjunct to total parenteral nutrition results in a net positive protein balance in critically ill patients.[38] On the other hand, in a multi-institutional, randomized, controlled trial of critically ill adults, the use of high dose rhGH resulted in longer length of hospitalization and a higher mortality rate.[39]

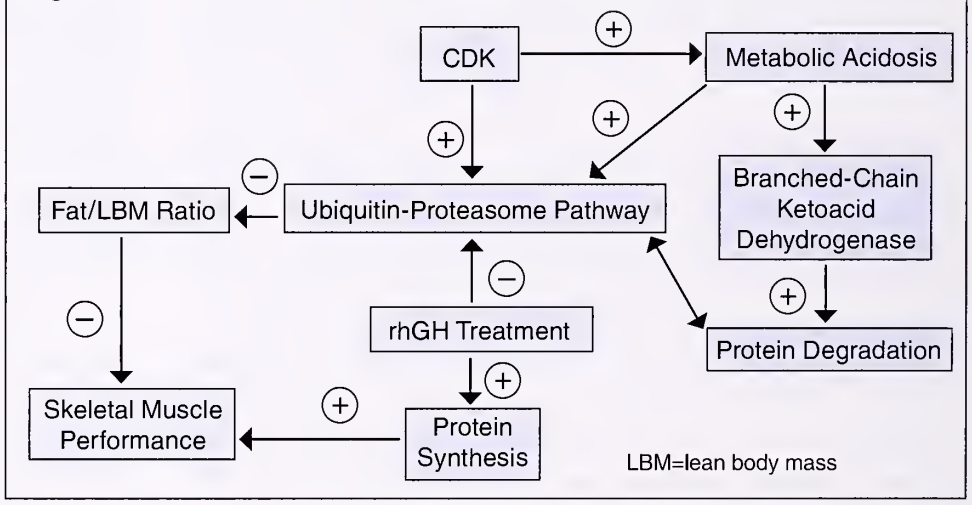


Figure 3 PROTEIN METABOLISM IN CKD: Effects of rhGH Treatment

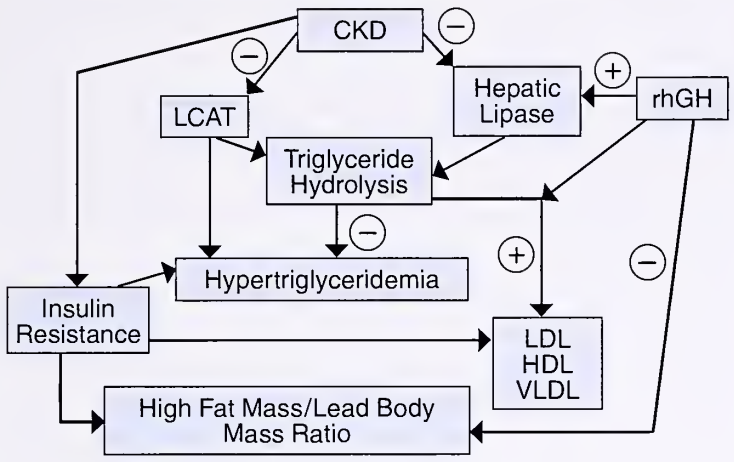
Lipid Metabolism in CKD

CKD subjects exhibit a reduction of lecithin-cholesterol acyl transferase (LCAT) enzyme, down-regulation of apo-A1 genes, and inhibition of hepatic lipase activity.[9] (Figure 4) Consequently, there is impaired hydrolysis of triglycerides (TG) in high-density lipoprotein (HDL), very low-density lipoprotein (VLDL), and intermediate-density lipoprotein (IDL), resulting in hypertriglyceridemia. Plasma low-density lipoprotein (LDL) has been shown to be elevated due to a down-regulation of its receptor function.[9] In addition, insulin resistance may promote dyslipidemia and pro-coagulant activity in CKD.[40] The pattern of lipid profiles in CKD patients are strikingly similar to findings

in metabolic syndrome. Both clinical syndromes share other characteristics such as hypertension, altered body composition, low-grade persistent inflammation, and hyperinsulinemia with a common outcome of premature cardiovascular (CV) disease.[41]

Figure 4

LIPID METABOLISM IN CKD: Impact of rhGH Treatment



LCAT=lecithin cholesterol acyl transferase; LDL=low-density lipoprotein; HDL=high-density lipoprotein; VLDLP=very low-density lipoprotein; ⊕=high; ⊖=low

rhGH Therapy and Lipid Metabolism

In general, rhGH therapy improves lipid profiles by decreasing LDL and apo-B while increasing HDL.[40] By induction of lipoprotein lipase and stimulation of LDL receptor, rhGH attenuates the characteristic increase in VLDL-TG in CKD.[40] In addition, rhGH reduces visceral adiposity, increases lean body mass, and restores normal body composition in CKD.[42] However, it is yet to be seen if these favorable metabolic and biological changes will translate into a better long-term CV outcome in CKD. On the other hand, GH therapy may increase lipoprotein (a), an independent CV disease risk factor.[40] While it shares a common lipid fraction with LDL, lipoprotein (a) clearance is not influenced by the GH-induction of LDL-receptor activity.[40] Nevertheless, the clinical significance of the modest yet notable increase in lipoprotein (a) during rhGH treatment on CV health is not known.

FOOD INTAKE AND ENERGY HOMEOSTASIS

Uremia promotes excessive transport of tryptophan across the blood-brain barrier and consequently increases neuronal synthesis of serotonin, an endogenous anorectic compound.[43] Adequate food intake may be further compromised in uremic patients by an accumulation of cholecystokinin, TNF-α, interleukin (IL)-1, leptin, and middle molecule toxins (eg, beta (2)-microglobulin, advanced glycation end products).

Ghrelin and rhGH in CKD

Ghrelin, an endogenous ligand for GH secretagogue-receptor, is principally secreted by pancreatic alpha-like cells (designated Gr cells) from the stomach fundus, in response to changes in nutritional status.[44] In addition to a potent pituitary stimulation for GH secretion, ghrelin increases food intake by activating agouti-related peptides and neuropeptide Y within the hypothalamus.[45] Experimental use of ghrelin in human subjects was shown to increase food intake, energy consumption, and visual analog scores for appetite.[46] Although the physiological consequence is unknown, there is often accumulation of biologically active (acylated polypeptide) and inactive (desacyl) ghrelin in CKD subjects because of impaired renal clearance. It may be speculated that ghrelin retention constitutes an adaptive mechanism to promote caloric intake in chronic uremia. Perhaps ghrelin's failure to correct the calorie deficiency state arises from the prevailing end-organ resistance to its orexigenic (appetite-stimulating) effects. Similarly, its role in promoting appetite may be physiologically counteracted by the anorexic forces from excessive accumulation of leptin, serotonin, and cytokines in CKD. It has yet to be determined whether the use of ghrelin as an adjunct to rhGH might be beneficial in overcoming anorexia in chronic uremia.[45]

It has been suggested that there may be a negative feedback control of ghrelin by the GH/IGF-I axis. Thus, a short-term rhGH induction of IGF-I causes a proportionate reduction in ghrelin with no alteration in plasma adiponectin.[47] On the other hand, a reduction in body fat mass from long-term use of rhGH may contribute to an increase in circulating levels of ghrelin and adiponectin.[47] The confounding effect of impaired filtration and/or catabolism of ghrelin in renal failure on the purported ghrelin-GH/IGF-I feedback axis is not known.

Leptin and rhGH in CKD

Hyperleptinemia is a common finding in renal failure, and may result from decreased renal clearance, increased secretion from adipose tissue, and hyperinsulinemia. Leptin is a potent endogenous anorexic agent; its effect may be modulated by rhGH therapy. Thus, administration of rhGH in the Zucker obese rat (which is characterized by leptin and insulin resistance) induces lipolysis and down-regulates leptin gene expression in visceral fat mass.[48] However, as previously stated, the appetite-promoting effect of rhGH may be overcome by persistent hyperleptinemia in CKD subjects. Recent discovery of leptin receptor isoforms in multiple organs suggests that leptin is an important mediator of other unknown biological functions.[49] Therefore, further studies are required in defining the roles of leptin in the modulation of metabolic and nutritional derangements in uremic syndrome.

SLEEP DEFECTS AND rhGH

About 50% to 70% of adults with end-stage kidney disease suffer from sleep apnea, insomnia, daytime somnolence, and restless leg syndrome.[50] In CKD the

high prevalence of sleep disorders may be confounded by co-morbidities of obesity and depression. However, there is often a strong positive correlation between blood urea nitrogen and indices of sleep dysfunction in patients with kidney failure.[50] Potential complications of sleep defects in uremia may include resistant hypertension, autonomic dysfunctions, and left ventricular hypertrophy.[51] Corroborating the role of uremic burden in sleep dysfunction is the remarkable improvement in symptoms with the administration of daily nocturnal hemodialysis. To date, there are no studies in humans on the therapeutic role of rhGH on sleep defects in CKD, although rapid eye movement (REM) sleep is restored by rhGH, and non-REM sleep is modulated by GHRH in GH-deficient (transgenic) animal models.[52] Similarly, use of ghrelin, a GH secretagogue, results in a preponderance of the more physiological pattern of slow and delta waves that occur during sleep.

Although there are case reports of sudden deaths from obstructive sleep apnea attributed to the use of rhGH in patients with Prader-Willi syndrome, scientific analysis has failed to confirm these assumptions.[53-55] On the contrary, there is potential for beneficial effects on respiratory physiology because of the favorable effects of rhGH on inspiratory drives, ventilatory muscle functions, respiratory quotients and resting energy expenditure.[56-58]

IMMUNE FUNCTION

CKD is characterized by a persistent micro-inflammatory state with increased circulating levels of IL-1, IL-6, and TNF-α cytokines. Negative nitrogen balance may result from the reduced hepatic syntheses of albumin and apo-lipoprotein; however, increased release of fibrinogen and amyloid precursors by the liver may enhance vascular thrombogenicity.[9]

Immune deficiency in CKD results from a direct inhibition of uremic toxins and/or altered metabolic activities of immunological cells, including neutrophils, lymphocytes, and macrophages. One subset of T-helper cells, Th-1, is the effector of cell-mediated immunity and recruits new Th-1 cells by producing interferon-gamma while inhibiting Th-2 induced cellular differentiation.[59] The other subset of T-helper cells, Th-2, secretes inhibitory IL-4 and IL-10 cytokines and consequently attenuates the self-perpetuation of Th-1 cells. Uremia shifts the delicate regulatory balance between Th-1 and Th-2 cellular pathways in favor of the latter, thereby causing a depression of cell-mediated immunity.[59] In addition, the impaired expression of B7-2 (co-stimulatory) molecules on the surface of antigen-presenting cells may weaken activation of effector T cells.[60]

The capacity for B-cell antibody production and superoxide generation by polymorphonuclear leukocytes are also reduced in a uremic milieu.[9] The defect may be due to elevated cytosolic Ca^{2+} resulting in poor ATP generation (impaired mitochondrial oxidative phosphorylation) and may be reversed by calcium-channel blockers.[9] Increase in neutrophil apoptosis is in part mediated by the Fas-Fas-L pathway in CKD; there is a positive correlation between Fas-mediated apoptosis and creatinine clearance in plasma obtained from uremic subjects.[61]

rhGH Impact on Immune Dysfunction

GH stimulates T-cell cytotoxicity and releases superoxide anion from inflammatory cells. CD4 and NK-cell activities were shown to be restored in GH-deficient adults treated with rhGH, while phagocytic function was normalized.[62] In addition, rhGH was shown to prevent apoptosis of immunologic cells by inactivating the pro-apoptotic Fas-FADD pathway and increasing the anti-apoptotic expression of Bcl-2. The overall physiological impact was a down-regulation of Caspase 3, an intracellular effector of apoptosis.[63]

GH is a member of the cytokine super-family and has a similar structure to granulocyte colony-stimulating factor.[64] GHRs, which bind to GH, are found on a number of immunological cell surfaces. Use of rhGH in severe sepsis may exacerbate the ongoing inflammatory process by cross-activation with other cytokine-receptors and, thereby result in a higher fatality rate.[65] In a rat model of bacterial sepsis, increased expression of suppressors of cytokine signaling (SOCS)-1 and -3 inhibited intracellular signaling of GHR, resulting in a poor generation of IGF-I.[66] Thus, a relative IGF-I deficiency may contribute to the impairment of glomerular filtration rate that may result from septicemia. Although in normal circumstances IGF-I increases renal perfusion, its administration in a rat model of ischemic renal failure results in higher mortality, apparently by evoking adverse inflammatory processes.[67]

The pro-inflammatory activity of rhGH was initially postulated to be a potential cause of allograft rejection. However, clinical evidence suggests otherwise, and the safety and efficacy of rhGH was recently demonstrated in renal transplantation.[68] In pediatric renal allograft recipients, rhGH has also been shown to prevent steroid-induced protein catabolism, maintain skeletal mass, and improve linear growth rate. In addition, postoperative administration of rhGH in rats with small bowel transplant restores morphology of allograft mucosa and promotes a net positive nitrogen balance.[69] Furthermore, the perioperative use of rhGH in immunocompromised rats enhances surgical wound healing.[70] Given that post-transplant use of the immunosuppressant sirolimus may cause a delay in wound healing because of its antifibrotic property, a study of the role of rhGH in this regard may provide useful information.

BONE MINERAL CONTENT AND rhGH

Within a few weeks of initiation of rhGH therapy, the molecule interacts with the bone-forming unit by increasing the biochemical markers of bone formation and resorption. In general, short-term (3–6 months) rhGH therapy may reduce or maintain bone mineral density, while treatment of GH-deficient adults for 2 years results in a sustained increase in mineralization.[71] On the other hand, the common use of high-dose calcium and calcitriol in CKD subjects for the treatment of hyperphosphatemia may result in suboptimal skeletal response to rhGH. Calcium-containing phosphate binders and vitamin D inhibit chondrocyte proliferation and delay mineralization, thereby causing adynamic bone disease.[72] Resistance to GH effects is manifested by low expression of IGF-I protein and decreased bone morphogenetic protein-7 staining, despite an increase in GH concentration and higher density of GHR.[72] It may therefore be prudent to avoid calcium-containing phosphate-binders and ensure appropriate vitamin D doses in CKD subjects receiving rhGH.[73]

There is evidence to suggest that GH may play a modulatory role in the musculo-skeletal effects of parathyroid hormone. Administration of rhGH to GH-deficient subjects improves end-organ responsiveness with a decrease in urinary calcium excretion, increased tubular phosphate reabsorption, and increased markers of bone turnover (type I collagen C-telopeptide and pro-collagen type I amino-terminal propeptide).[74]

QUALITY OF LIFE

Psychometric analysis and physical assessment of renal patients reveals a high prevalence of reactive depression, reduced physical performance, and cognitive deficits. However, psychosocial support, physical exercise, and anemia control may ameliorate many of these deficits. Administration of rhGH may also play a positive role as replacement therapy in GH-deficient adults; rhGH has been shown to improve quality-of-life indices.[75] Similarly, rhGH improves linear growth and physical agility, and reduces psychosocial burden in children with Prader-Willi syndrome.[76,77] Confounding variables such as anemia in CKD make studying the psychosocial impact of rhGH a difficult exercise.

CONCLUSIONS & SPECULATION

This review describes and highlights the potential therapeutic impact of rhGH in CKD patients. In the absence of kidney transplantation, it is important to restore the profound metabolic and physiological defects arising from renal insufficiency. In many instances, studies in GH-deficient models have demonstrated the beneficial effects of rhGH therapy beyond the longitudinal skeletal growth for which rhGH is commonly indicated. Additional problems in CKD patients for whom rhGH may play a significant role include modulation of nutritional inadequacies, altered body composition, immune dysregulation, and impaired sexual development and/or reproductive capacity. However, given the differences in their pathogeneses, it may be overly simplistic to project similar benefits of rhGH therapy to all the clinical settings of growth failure in CKD.

The multifaceted physiological effects of rhGH should still be taken into consideration in future studies of renal patients. Efforts must be made to broaden the scope of outcome measures to include cellular growth, cellular metabolism and function, neurocognitive development, psychosocial impact, sleep physiology, energy homeostasis, and anemia control. The beneficial role of rhGH in uremic cardiomyopathy, bone disease, anemia management, body composition, hospitalization requirements, and vascular diseases should also be examined. Co-morbidities are common in CKD and, therefore, multiple pharmacological agents are often needed to treat the disease. The physiological outcome of the combined use of erythropoietin, steroids, vitamin D, carnitine supplements, and other nutritional supplements with rhGH requires further study. Experimental studies in animals suggest a favorable role for rhGH in surgical wound healing; studies are therefore needed to examine the role of rhGH in ameliorating delayed wound-healing that may characterize the use of sirolimus after surgical transplantation.

Furthermore, the role of ghrelin (a recently discovered endogenous GH secretagogue) in CKD requires critical evaluation. Relevant questions for future studies are numerous. What is the role of ghrelin in food intake behavior in CKD patients? What are the metabolic effects of uremia on the capacity of Gr cells to produce ghrelin? What is the effect of uremia on the pituitary GH secretagogue receptor? What is the therapeutic impact of oral administration of ghrelin as a sole agent and/or combined therapy with rhGH/rhIGF-I, GH releasing peptides, exogenous IGFBP-3, and IGF-I analogs? What are the relationships between ghrelin, leptin, cytokines, and UCP polymorphism in the regulation of food intake, energy balance, and body composition in CKD?

Finally, the essence of this review is to inform the scientific community of the need for operational research endeavors concerning the metabolic impacts of rhGH therapy. Therefore, efforts must be made to critically assess the risk and benefit of the continued use of rhGH beyond the traditional end-point of linear skeletal growth in children with CKD. Hopefully, an improved understanding of the roles of rhGH in restoring physiological disturbances in CKD will provide added value to the treatment of such patients throughout their lives.

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ABSTRACTS FROM THE LITERATURE

Hypothalamic Amenorrhea and Leptin

The authors assessed the effects of leptin treatment in 8 patients with hypothalamic amenorrhea, compared with 6 patients who were not treated. All 14 patients had secondary amenorrhea for 6 months or longer, coincident with increased exercise or low body weight and were otherwise healthy without acne, hirsutism or LH/FSH, TSH, and prolactin alterations. Basal and follow-up assessments in a clinical research center included comprehensive endocrine, body composition, metabolic rate analyses, bone densitometry and pelvic ultrasonography. The patients treated with leptin (r-metHuLeptin) received 0.08 mg/kg/day subcutaneously for 2 to 3 months, with 40% of the dose given at 8:00 AM and 60% given at 8:00 PM. If patients ovulated the study was terminated at 2 months. If no ovulation occurred the dose was increase to 0.2 mg/kg/day for a third month. Leptin treatment increased mean LH levels and LH pulse frequency after 2 weeks of treatment and increased maximal follicular diameter, the number of dominant follicles, ovarian volume and estradiol levels over the study period. Three patients had ovulatory menstrual cycles; 2 had preovulatory follicular development and withdrawal bleeding during treatment. Leptin treatment significantly increased levels of free T_3, free T_4, IGF-I, IGFBP-3, bone alkaline phosphatase and osteocalcin but not cortisol, corticotropin, nor urinary N-telopeptide. Untreated control patients did not have any significant changes in any of these variables. Body weight did not change in the control patients; however it decreased slightly among the treated ones, owing to a small decrease in body fat without changes in lean body mass. No significant changes in metabolic rates or food intake occurred. The authors concluded that the relative leptin deficiency in women with hypothalamic amenorrhea is improved with leptin treatment. This results in improved reproductive, thyroid, growth hormone axis and markers of bone formation, suggesting that leptin is required for normal reproductive and neuroendocrine function.

Welt CK, Chan JL, Bullen J, et al. Recombinant human leptin in women with hypothalamic amenorrhea. *N Engl J Med* 2004;351:987-97.

Editor's Comment: *Hypothalamic amenorrhea, also called functional amenorrhea, is frequently seen in women who are athletic, underweight and/or stressed. It is usually preceded by irregular menses, weight loss or increase in physical activity and it is considered to be the result of energy deficiency. In non-athletic women of normal weight it may be associated with psychosocial stress also related to subtle deficits in calorie and macronutrient intake. The central energy-related hormone, leptin, is the common factor underlying the pathogenesis of this entity. The study by Welt et al adds data substantiating the importance of leptin in mediating the neuroendocrine abnormalities of hypothalamic amenorrhea, a leptin deficiency condition. They demonstrated an improvement with leptin treatment, without other medications to induce menstruation, while the patients maintained their usual dietary intake, exercise habits and lifestyle. However, let's not tread into new expensive treatments without correction of nutrient deficiencies or without first attempting to modify the dietary intake to meet all the energy and nutrient needs of the patient. The accompanying editorial by Ahima[1] addresses the distinguishing features of this condition from anorexia nervosa, as well as an erudite explanation of the pathophysiology of the disease as it relates to body fat, leptin and hypothalamic amenorrhea.*

Fima Lifshitz, MD

Reference

1. Ahima RS. *N Engl J Med* 2004;351:10:959-62.

Statin Therapy in Hypercholesterolemic Children

Wiegman and associates report findings of a 2-year randomized placebo-controlled efficacy and safety trial of pravastatin for the treatment of familial hypercholesterolemia in children ages 8 to 18 years. Two hundred fourteen children (100 boys), mean age 13.0 years, were studied. Inclusion criteria were: one parent with a definite clinical or molecular diagnosis of familial hypercholesterolemia; at least 3 months on a fat-restricted diet (<30% of total calories from fat, 10% saturated fat); 2 fasting LDL-C levels of at least 155mg/dL; no current drug treatment or use of plant sterols. The primary efficacy variable was change from baseline of carotid intimamedia thickness (IMT) as measured by ultrasound. Blood samples were measured for total cholesterol, HDL-C, LDL-C and triglycerides at 3–6 month intervals over the 2-year study. In addition, ALT, AST, and CPK were measured for safety reasons, and levels of sex steroids, gonadotropins, cortisol, and TSH were determined to survey for potential side effects of the drug on growth and sexual development. Height and weight were measured and Tanner staging was performed at baseline, 1, and 2 years.

Baseline characteristics were similar in both groups. The mean carotid IMT was attenuated after 2 years of treatment, while there was a trend towards an increase in the placebo group. The overall change between the 2 groups was statistically significant. LDL-C levels were reduced in the treatment group, while HDL-C, triglyceride and lipoprotein(a) levels remained unchanged. All hormone levels (corticotropin, cortisol, LH, FSH, DHEA-S, TSH, estradiol, testosterone) were similar in both groups at 2 years. Height and weight increased similarly in both groups, as did stages of sexual development. AST, ALT, and CPK levels were also similar, although one child in the placebo group had an asymptomatic but marked increase in CPK, which returned to normal.

The authors point out that this is the first long-term safety and efficacy trial of a 3-hydroxyl-3 methylglutaryl coenzyme A reductase inhibitor (statin) in children with familial hypercholesterolemia. The drug was both effective and well tolerated with minimal observable side effects. There were no effects on growth or sexual development. Despite these encouraging findings, they caution that even longer studies are needed to establish the safety of this class of drugs.

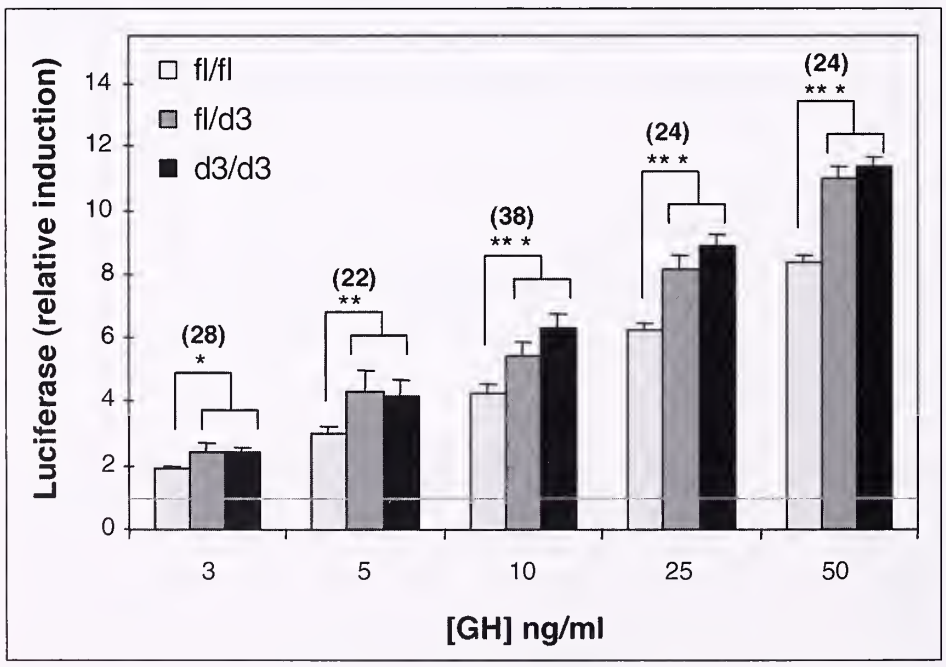
Wiegman A, Hutten B, de Groot E, et al. Efficacy and safety of statin therapy in children with familial hypercholesterolemia: a randomized controlled trial. *JAMA* 2004;292: 331-7.

Editor's Comment: *This is a welcomed study. Although the efficacy of statins in reducing LDL-C has been reported in several studies, this safety study is reassuring. More and more frequently pediatric endocrinologists are faced with younger and younger children with obesity and hypercholesterolemia, or diabetes and hypercholesterolemia, and need to recommend effective and safe therapy. Diet and exercise, unfortunately, are rarely practiced with sufficient adherence to be considered effective and realistic treatment options. Furthermore, resins are poorly tolerated in this age group. The use of statins is therefore an obvious therapeutic choice, but information regarding their long-term safety and side effects has been lacking. We would encourage these authors to continue their study of these children with the anticipation that further data will establish safety over an even longer time period.*

William L. Clarke, MD

Growth Hormone Receptor and Responsiveness to Growth Hormone

Intrigued by the clinical observation that the linear growth response to a similar dose of growth hormone (GH) in GH deficient (GHD) and in non-GHD children with idiopathic short stature (ISS) or children with intrauterine growth retardation (IUGR) varied substantially, the investigators correlated the biological effectiveness of recombinant human GH (rhGH) with 2 known isoforms of the GH receptor (GHR). The human gene GHR consists of 9 coding exons with exons 3–7 encoding the extracellular domain of 246 amino acids; there is one full-length isoform of GHR and a second isoform in which the 22 amino acid sequence coded by exon 3 is omitted by alternative splicing during transcription (d3-GHR). In prepubertal children with ISS or IUGR (defined by short birth length), the frequency of the d3-GHR isoform was comparable to that of normal subjects. The GHR genotype (GHR/GHR, GHR/d3-GHR, d3-GHR/d3-GHR) did not affect basal growth rate. When treated with rhGH, subjects with at least one d3-GHR isoform grew more rapidly in response to a standard dose of rhGH (0.36 or 0.23 mg/kg/week in 2 separate trials) than did those with the GHR/GHR genotype during the first 2 years of therapy. There was no difference in growth response to rhGH between children with 1 or 2 d3-GHR alleles or between those with ISS or IUGR. Expression of the GHR and d3-GHR isoforms in HEK fibroblasts *in vitro* demonstrated that in response to hGH the transcriptional activity of the luciferase reporter gene was ~30% greater in cells with d3-GHR than GHR (Figure). The authors concluded that analysis of the GHR genotype may permit more appropriate individualization of rhGH dosage (pharmacogenetic dose selection) in clinical conditions in which administration of rhGH is appropriate.



In vitro bioactivity of full-length GHR and d3-GHR. HEK 293 cells transiently expressing full-length GHR, d3-GHR or both were stimulated by increasing concentrations of GH for 8 h. Relative induction of LHRE-luciferase reporter gene is expressed relative to unstimulated cells (value of 1, horizontal line).
Number of experiments in ( ), *P < 0.005, ** P < 0.0005, ***P < 0.00001
Reprinted with permission from: Dos Santos C, Essioux L, Teinturier C, Tauber M, Goffin V, Bougnères P. *Nat Genet* 2004. 36:720-4 Copyright © Elsevier. All rights reserved.

Dos Santos C, Essioux L, Teinturier C, Tauber M, Goffin V, Bougnères P. A common polymorphism of the growth hormone receptor is associated with increased responsiveness to growth hormone. *Nat Genet* 2004. 36:720-4.

Editor's Comment: *The mechanism(s) by which the shorter d3-GHR transmits a more potent signal in response to ligand bind than does the full-length GHR is not known. The 22 amino acid sequence of exon 3 is not near the interface of ligand and receptor, and the mechanism by which its loss leads to increased receptor activity is unknown at present. It does not affect hGH/GHR binding or internalization. The d3-GHR polymorphism might permit more rapid propagation of signal to the intracellular signal transduction systems that mediate the cellular responses to hGH. In this regard, it would be of interest to study the dynamics of this system in cells expressing either the full-length or shortened GHR isoforms. The report also raises the question that if a polymorphism that increases responsiveness to hGH exists, might there not also be a subtle polymorphism that mildly depresses GHR transduction of the hGH signal? Might this be another pathway through which the "genetic" regulation of growth and adult stature is mediated?*

Allen W. Root, MD

Therapeutic RNAi for Genetic Skeletal Disease?

RNA interference (RNAi) is a gene silencing phenomenon first identified in the nematode, *C. elegans*, but was subsequently found to occur in higher organisms including humans. It probably evolved as an ancient defense mechanism for cells to fend off mobile genetic elements, such as RNA viruses and transposons, but today it has been implicated in a growing number of cellular processes.

As discussed by Stevenson, RNAi involves sequence specific degradation of target RNAs triggered by the formation of double stranded RNA (dsRNA). When it occurs naturally, long dsRNA is processed to short interfering RNAs (siRNAs) 21-24 bases in length by a dsRNA-specific endonuclease named Dicer (Figure). They are incorporated into a nuclease complex referred to as the RNA-induced silencing complex or RISC. Unwinding of the siRNAs activates and directs RISC to the target RNAs, which are cleaved and degraded. The complementarity between the siRNA and the target RNA determines the sequence specificity of RNAi.

An important advance in the RNAi field was the discovery that exogenous synthetic siRNAs or endogenously synthesized siRNAs driven by viral vectors could be incorporated into RISC and induce sequence-specific degradation of target RNAs. This created an extremely powerful tool for scientists to "knock down" expression of genes of interest simply by adding synthetic RNA duplexes to the medium of cultured cells, introducing viral vectors that express siRNAs into cells or even generating transgenic animals that synthesize siRNAs.

RNAi is much more complex than outlined here, and there are many technical difficulties that complicate the use of RNAi to knock-down gene expression in experimental systems. Nevertheless, RNAi has stimulated considerable interest in the pharmaceutical/biotech industry as a potential therapeutic agent for human disease. The best examples to date have to do with treatment of infectious diseases, such as those caused by HIV, hepatitis viruses and poliovirus, as well as cancers that are mediated in part by overactive oncogenes. In the case of viral infections, interfering RNAs could be targeted to viral transcripts required for viral replication or survival. In the second case, using RNAi to silence expression of BCR-ABL, the fusion gene that results from the Philadelphia chromosome translocation in chronic myelogenous leukemia or mutated RAS oncogenes that drive several types of cancer would be appealing.

Receiving less attention to date, but of probably at least as much interest to readers of *GGH*, is the potential use of RNAi to knock down expression of mutant alleles in dominantly inherited genetic disease. In concept, siRNAs could be tailored to distinguish mutant from normal (wild type) alleles and block only mutant allele expression. This could convert a dominant negative disorder, ie, a disorder in which the product of the mutant allele interferes with the function of the normal (wild type) allele product, to a disorder that results from haploinsufficiency or functional loss of one allele. For families in which both forms occur, manifestations are usually milder in the form resulting from haploinsufficiency, ie, osteogenesis imperfecta type I – haploinsufficiency vs osteogenesis type II – dominant negative. Thus, there is potential benefit from this therapeutic strategy.

Despite the excitement and promise of therapeutic RNAi, there are many obstacles, the greatest of which is delivery. Systemically delivered siRNAs face degradation by nucleases, and the use of viral vectors to target organs of interest is still in its infancy. A recent publication by Soutscheck and colleagues provides evidence that chemically modified siRNAs can successfully knock down endogenous genes in living mice. More specifically, they targeted expression of the gene encoding apoprotein B (*apoB*) in the mouse liver and jejunum where it is known to be expressed at high levels with 2 siRNAs known to silence *apoB* in cultured cells. They modified the *apoB* siRNAs by chemically stabilizing their backbone and also by adding cholesterol to their 3' end. The modified siRNAs were then compared to unmodified *apoB* siRNAs and other controls.

The results showed that the cholesterol-conjugated *apoB* siRNAs were significantly more stable in serum than their unconjugated counterparts. When administered intravenously, one of the conjugated *apoB* siRNAs was very effective at lowering *apoB* mRNA and *apoB* protein levels, as well as total cholesterol and LDL cholesterol. They observed no evidence of "off-target" effects, that is, effects attributed to silencing of genes other than *apoB* or other obvious complications from the injections. The authors concluded that exogenously administered chemically

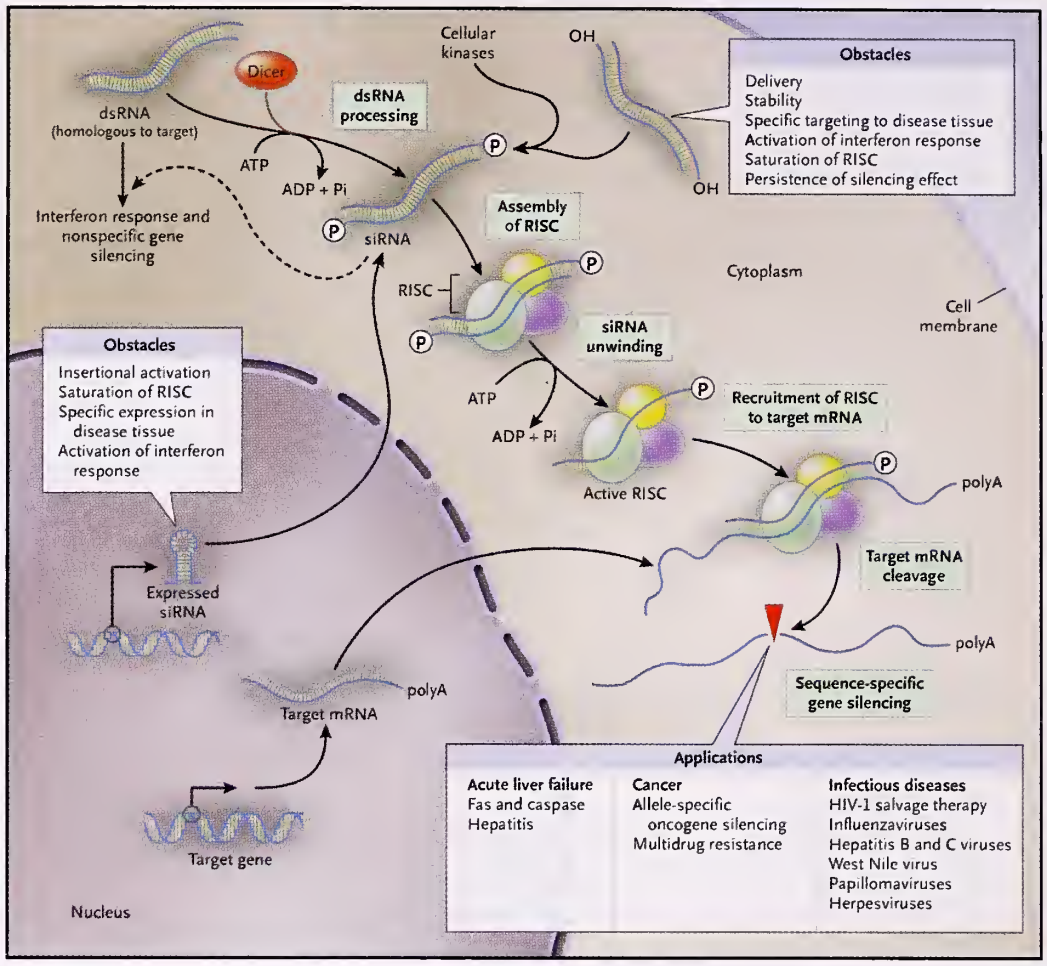
modified siRNAs can potentially be used to silence expression of endogenous genes involved in human disease.

Stevenson M: Therapeutic potential of RNA interference. *N Eng J Med* 2004;351:1772-7.

Soutschek J,Akinc A, Bramlage B, et al. Therapeutic silencing of an endogenous gene by systemic administration of modified siRNAs. *Nature* 2004;432:173-8.

Editor's Comment: *RNAi has had a major impact on science since its relatively recent discovery. It is still not entirely clear how it works and there remain concerns about specificity and the so-called off target effects on genes other than specifically targeted genes. Nevertheless, it has great promise as a means to treat not only cancer and infectious diseases, but genetic diseases in which mutant alleles differing from their normal alleles by only a single base can be specifically targeted. It will probably be years before such treatment becomes realistic for humans, but the success of substantially knocking down apoB expression by systemically administering chemically modified apoB siRNAs in mice is very encouraging. One note of caution is that the growing skeleton may be difficult to target because the cartilaginous growth plate is relatively avascular compared to most tissues such as liver and gut.*

William A. Horton, MD



Mechanism of Gene Silencing by RNA Interference. The double-stranded RNA (dsRNA) is processed and assembled into the RNA-induced silencing complex (RISC) and subsequently incorporated into target mRNA for the sequence-specific gene silencing application.

Variation in Expression in Human Genes

Medical genetics textbooks typically distinguish between continuous and discontinuous variation in (clinical) phenotype. The latter can often be traced to a single change in the DNA sequence of a gene, ie, a "mutation" that serves as the basis of classic mendelian disorders. The genetic basis of continuously variable traits, such as height or blood pressure, is more difficult to explain. Variation in baseline expression of genes represents a mechanism that could contribute to continuous phenotypic variability. It is known to exhibit familial aggregation suggesting that it is heritable, but the tools to study the genetics of variation in human gene expression have only recently made it feasible to explore this notion. Morley and colleagues now document the existence of regulators of baseline gene expression.

The investigators utilized microarray technology to measure expression levels of genes, which they refer to as "gene expression phenotypes," in immortalized B cells from members of 94 Center d'Etude du Polymorphism Humain (CEPH) Utah families. Starting with approximately 8500 genes active in these cells, they found 3554 genes that showed greater variation of expression between individuals than between replicates from the same individual. They then carried out genome-wide linkage analysis using single nucleotide polymorphisms to identify the genetic determinants of this variation. The results showed that variation in expression of 984 genes was genetically linked to one or more regions of the genome.

They assumed that regions linked to expression levels were regulatory regions or "regulators". They examined the spatial relationship of the regulators to the 142 "target" genes that exhibited the strongest evidence for linkage. Twenty seven (19%) mapped to within 5 Mb of the target gene; they considered these to be *cis*- acting regulators because of their relatively close proximity to the coding sequence of the target gene. One hundred ten (77.5%) mapped further away and were designated *trans*-acting regulators. Both *cis*- and *trans*-acting regulators were

found for 5 (3.5%) of the variably expressed genes. Many of these genes (164/984, or 16%) had multiple regulators of expression.

In addition to genomic regions containing regulators that influence single expression phenotypes *in cis* or *in trans*, the authors also found genomic regions that contained transcriptional regulators of multiple expression phenotypes. To further characterize these regulators, they divided the genome into 5 Mb windows and searched for regulatory "hotspots" within these windows. Two hotspots were detected, one of which mapped to chromosome 14 (14q32) and the other to chromosome 20 (20q13). Further analysis showed that these 2 regulatory hotspots influence expression of 31 of the 984 target genes under investigation. The authors suggest that their existence provides evidence for master regulators of baseline gene expression in humans.

Finally, they asked if differential expression of target gene alleles could be explained by *cis*-acting regulators. Analysis of individuals in whom alleles could be distinguished by single nucleotide polymorphisms showed that some of the variable expression could be attributed to the influence of the *cis*-acting regulators.

Morley M, Molony CM, Weber TM, et al. Genetic analysis of genome-wide variation in human gene expression. *Nature* 2004;430:743-7.

Cox NJ. An expression of interest. *Nature* 2004;430:733-4.

Editor's Comment: *This paper reminds us that the level of expression is an important aspect of gene action. Reduced or increased gene expression can influence quantitative traits, such as height. One can also envision a situation in which a mutation in a trans-acting regulator could cause disease by decreasing or increasing expression of its target gene(s). Take osteogenesis imperfecta type I for example; it typically results from mutations that cause transcripts from a mutant COL1A1 allele to terminate prematurely or undergo nonsense-mediated mRNA decay, functionally inactivating one of the 2 COL1A1 alleles. It is conceivable that a loss of function mutation of a trans-acting regulator of this locus could produce a similar adverse effect on type I collagen synthesis, especially if it were homozygous. Of note, such a mutation would not show linkage to the COL1A1 locus. There are several limitations of this investigation as noted by the authors and an accompanying news and views article. For instance, mRNA levels are only one determinant of the level of protein encoded by a given gene. Gene expression differs in different tissues, at different developmental stages and in response to physiologic and pathologic factors that are probably not reflected in immortalized B cells.*

William A. Horton, MD

IGF-I Receptor Signaling: Mechanisms of Growth Stimulation

Wu and colleagues used 2 cell models to study the effects of insulin-like growth factor-I receptor (IGF-IR) signaling via insulin receptor substrate (IRS)-1 on the upstream binding factor 1 (UBF1), a regulator of ribosomal RNA (rRNA) synthesis. 32D cells (myeloid cells dependent on interleukin-3 (IL-3) for growth) express neither IRS-1 nor IRS-2. In complement, mouse embryo fibroblasts (MEFs) express IRS-1 but have a targeted disruption of the IGF-IR gene (R^- cells).

Apoptosis normally takes place in 32D cells upon removal of IL-3. 32D cells expressing IGF-IR (32D IGF-IR cells) continue growing for 48 hours after IL-3 is replaced

with IGF-I, and then undergo granulocyte differentiation. 32D IGF-IR cells ectopically expressing IRS-1 grow indefinitely without differentiation. R^-cells were also used to develop sister cells for comparison. R^-/T cells express the SV40 large T antigen, while R^+ cells have the IGF-IR reintroduced. IRS-1 is mostly nuclear in IGF-I-stimulated R^+ cells and in R^-/T cells, but cytoplasmic in the parental R^- cells.

Using these 2 systems, the authors showed that IGF-I increased transcription from the rDNA promoter (ie, activated UBF1) in a time course compatible with nuclear translocation of IRS-1. Since UBF1 activation generally occurs via phosphorylation, additional experiments showed that UBF1 phosphorylation, mainly in the C terminus, was IGF-I stimulated and IRS-1 dependent. Beyond that, UBF1 regulation in the 2 cell models differed. In the myeloid cells deprived of IL-3, 32D IGF-IR/IRS-1 cells died without IGF-I, but maintained high levels of UBF1 protein when stimulated with IGF-I. The 32D IGF-IR cells (ie, without IRS-1) had high UBF1 protein levels, which dropped at 48 hours (ie, while the cells were still growing exponentially and not yet showing any morphologic signs of differentiation) and completely disappeared by the time the cells were differentiated into granulocytes. The drop in UBF1 protein was due to both decreased synthesis and increased degradation, though UBF1 mRNA levels remained unchanged. In the MEFs, cells that do not differentiate, UBF1 protein levels were stable after IGF-I treatment in both R^+ and R^- cells. Thus, the authors concluded that IGF-IR/IRS-1 signaling regulates UBF1 activity, and hence the rDNA promoter, through phosphorylation and in some cells, through changes in protein level. UBF1 protein loss may be related to the differentiation process, which tends to involve nucleolar dissolution.

Wu A, Tu X, Prisco M, Basergo R. Regulation of upstream binding factor I activity by IGF-I receptor signaling. *J Biol Chem* 2005; 280:2863-72.

Editor's Comment: *IGF signaling through the IGF-IR is understood to stimulate cellular survival and proliferation, and at the systemic level, growth. IGF-IR is a tyrosine kinase that is activated by ligand binding. Phosphorylation of tyrosine residues in IGF-IR recruits adaptor molecules like IRS-1 that then start kinase cascades, most notably the PI3 kinase/Akt pathways and the MAP kinase pathway (for reviews, see References 1-2). The paper by Wu et al adds another mechanism whereby IGF-IR signaling stimulates growth: activation of UBF1 through nuclear translocated IRS-1 and presumably PI3 kinase. UBF1 regulates RNA polymerase I activity at the rDNA promoter, thereby regulating the rate of ribosome biogenesis. Because ribosomes are required for protein synthesis, proliferating cells invest much energy in ribosome generation (reviewed in Reference 3). Without concomitant synthesis, proliferating cells would only become progressively smaller. Thus, growth involves increasing numbers of cells with maintenance of proper cell size, and IGF-IR is involved in regulating both these processes.*

Adda Grimberg, MD

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Long-term Effects of Estrogen Treatment on Fertility in Tall Girls

Venn and colleagues identified from medical records 1248 Australian women who had been assessed and/or treated with estrogens (3mg DES or 150µg ethinyl estradiol daily) for tall stature during the years 1959 to 1993, to assess the effects of this treatment on long-term fertility. A group of 184 self-referrals (members of Tall Girls Inc an Australian advocacy group) were included in the study. To be included subjects had to have had a bone age determination at the time of assessment. Subjects were invited to complete a written questionnaire and computer-assisted telephone interview. The interview included questions regarding reproductive history including whether or not they had ever seen a doctor due to difficulty becoming pregnant, whether they had ever tried unsuccessfully for more than 12 months to become pregnant, and whether or not they had ever taken fertility drugs as treatment for infertility. The time to pregnancy was analyzed for each month of attempting pregnancy. Data from the medical records included age at menarche, treatment type, duration of treatment, and first and last assessment of estimated mature height by Bailey and Pinneau method.

The final sample size included 618 women (75% of the treated and 95% of the untreated). The mean age of these women was 39.8 years (treated) and 37.7 years (untreated). Both groups were similar in terms of marital status and highest level of education. Self-reported current height was greater in the treated women (179.0cm vs 176.8cm). Both groups were similar in terms of history of smoking, oral contraceptive use, age of first sexual intercourse and lifetime number of male sexual partners. There were no differences between the women treated with DES or ethinyl estradiol on any parameter. Women who had been treated with estrogen were more likely to report problems with fertility. When the data were adjusted for age, the women who had been treated were less likely to have ever been pregnant and to have ever had a live birth. Treated women were more likely to have tried unsuccessfully for 12 months to become pregnant, to have seen a doctor because of difficulty becoming pregnant, and to have taken fertility drugs. Height was not related to fertility problems and the differences between

the 2 groups remained when the self-referred women were excluded from the analysis. A significant, but weak duration of treatment effect was observed.

The authors state that the data were not sufficient to establish a pathophysiological cause for the reduced fertility. They also state that the likelihood of ever becoming pregnant and having a live birth, although statistically reduced for women who had been treated for tall stature, was only slightly lower than that for untreated women and that newer treatments for infertility may reduce that difference.

Venn A, Bruinsma F, Werther G, et al. Oestrogen treatment to reduce the adult height of tall girls: long-term effects on fertility. *Lancet* 2004;364:1513-8.

Editor's Comment: *Clearly there has been a significant drop in the number of girls seeking treatment to reduce mature height potential over the past 20 years. However, the authors note that a recent survey of pediatric endocrinologists in the United States reveals that 23% have treated such girls over the past 5 years. Thus, although the absolute number of girls seeking treatment is low, such treatment is still being sought and is available. The current study, although not the first to show the possibility of adverse reproductive effects of estrogen treatment for tall stature, is perhaps the largest long-term follow-up to date. The information is interesting and important. Pediatric endocrinologists need to be able to discuss these facts with each family seeking to reduce their daughter's mature height potential. It is reassuring that no obvious safety concerns were identified through these interviews and chart data.*

William L. Clarke, MD

Micropenis: Long-term Follow-up

These authors report the long-term outcomes of 46,XY males with micropenis, but no other genital deformity, identified and treated intermittently with androgens or hCG during infancy, childhood and/or adolescence. Lee and Houk determined adult stretched penile length (SPL) and social adjustment in 20 patients with SPL <–2 SD of normal at initial examination: 11 had hypogonadotropism and 3 primary testicular failure; in 6 patients no cause of the micropenis was identified. SPL increased in all subjects; adult SPL was >–2 SD of the adult mean in 14 subjects and between –2.5 and –2 SD in 4; 2 patients had adult SPL <–2.5 SD of the mean. Among these 20 patients and another 2 with micropenis first evaluated as adults, 21/22 were heterosexual; 8 were/had been involved in long-term heterosexual relationships. Relative to age-matched control subjects, those with micropenis (N=12 studied) had comparable findings in regard to heterosexual dating and sexual functioning, male friendships, education, employment, sports/leisure activities; none had a psychiatric illness. Despite normal adult SPL, 5 primarily obese patients stated that their penises were small. The investigators concluded that in adult men who had micropenis as children/adolescents: 1) 90% had adult SPL within the broad range of normal; 2) there was "reasonable social adjustment," no psychological pathology, and gender-appropriate sexual functioning.

Husmann evaluated adult SPL in 20 men with micropenis (here defined as SPL <–2.5 SD of normal) diagnosed and treated during infancy in whom SPL did not increase appreciably despite multiple courses of testosterone. Five patients had a mutation in the androgen receptor, 6 had hypogonadotropism, and 9 had no known cause of the micropenis. Mean pretreatment SPL was –3 SD (range –5.5 to –2.6) for age/race and mean adult SPL was –3.4 SD (range –5.9 to –2.2). All patients considered their penises to be small, and 5 had undergone (unsatisfactory) surgery to enlarge their penises; 19/20 were heterosexual; 12/20 men were sexually active, but 4 were incapable of vaginal penetration; 5 patients had mental illnesses requiring professional therapy. Despite these findings, Husmann concluded that these patients accept a male gender identity and many engage in a "satisfying heterosexual relationship."

Lee PA, Houk CP. Outcome studies among men with micropenis. *J Pediatr Endocrinol Metab* 2004. 17:1043-53.

Husmann DA. The androgen insensitive micropenis: Long-term follow-up into adulthood. *J Pediatr Endocrinol Metab* 2004.17:1037-41.

Editor's Comment: *In the report of Lee and Houk, in 5/20 patients (1 hypogonadotropic subject, 1 with primary testicular failure, and 2 with "idiopathic" micropenis) SPL SD score did not appreciably increase between diagnosis and adulthood, but these subjects are not specifically discussed further, and their psychosocial status is unknown. It would have been of interest if Husmann had also reported his experience with the outcome of patients with micropenis responsive to testosterone. These data are reassuring in that they further demonstrate that there is no basis to consider sex reversal in the 46,XY male with micropenis as their gender identity is firmly masculine. Furthermore, with current surgical procedures for penile reconstruction, the opportunity for satisfactory penile enlargement has improved substantially.*[1]

Allen W. Root, MD

Reference

1. Jordan GH, Rosenstein DI, Gilbert D. *Growth Genet Horm* 2002;18:33-8.

Growth Hormone Sensitivity in Obesity

These authors sought to explore the observation that insulin-like growth factor (IGF)-I levels remain normal in obesity despite reduced growth hormone (GH) levels. Ninety-one healthy adults (mean age about 50; range 21-82 years) were subdivided by body mass index (BMI) and gender; there were 19 normal weight men, 23 normal weight women, 15 obese men and 34 obese women (obesity defined as BMI > 30). Fat mass and percent body fat were measured by bioimpedance. GH sensitivity was assessed by an IGF-I generation test, with IGF-I levels measured before and 24 hours after a single, standard 7mg dose (21IU) of GH. The increment in IGF-I was greater in obese than normal-weight equivalents, negatively correlated with baseline IGF-I concentration, positively correlated with GH binding protein (GHBP) level, and seen in both men and women (pre- and post-menopausal). GHBP concentrations were higher in obesity, and also correlated with BMI, fat mass and percent body fat. The authors concluded that their study provides evidence of increased GH sensitivity in obesity. The fact they used a single, standard GH dose makes the result cleaner than earlier studies that employed a weight-based GH dosing scheme; IGF-I levels were higher in obese subjects, but in those studies, the obese subjects also received a greater GH dose. Because GHBP is the extracellular domain of the GH receptor (GHR), it is sometimes used as an indirect measure of GHR number. The finding of a positive association between GHBP level, markers of obesity and IGF-I increment led the authors to hypothesize that the enhanced GH sensitivity of obesity may be due to increased GHR density, itself resulting from the lower GH levels. Because the data are all associative, further studies are needed to test this hypothesis.

Gleeson HK, Lissett CA, Shalet SM. IGF-I response to a single bolus of growth hormone is increased in obesity. *J Clin Endocrinol Metab* 2005;90:1061-7.

Editor's Comment: *This paper clearly showed increased hepatic sensitivity to GH in obesity, at least in terms of IGF-I generation, which helps to explain the discordance between the low GH but normal IGF-I levels seen in obesity. The pediatric correlate of this adult study is the enhanced growth frequently experienced by obese children who continue growing despite GH deficiency (classically, craniopharyngioma patients who develop hypothalamic obesity and GH deficiency); the growth without GH phenomenon is reviewed in Reference 1. Proposed mechanisms include hyperinsulinism-stimulated growth, decreased IGFBP-1 levels resulting in increased bioavailable (free) IGF-I, and increased growth plate stimulation by sex steroids (increased aromatization by the greater adipose mass). An interesting finding came from studies of a model of endochondral ossification, the chondrocyte population of the skeletal growth centers in the mouse mandibular condyle. The growth center chondrocytes expressed leptin receptors and when stimulated by leptin, increased expression of IGF-I receptor, increased both proliferation and differentiation processes, and had larger growth plate growth.[2] Furthermore, when mice were calorie-restricted by 40%, circulating IGF-I levels dropped by 70% and tibial growth decreased by 5%; leptin treatment corrected the growth deficit despite further reductions in circulating IGF-I levels.[3] Thus, the growth-promoting consequences of obesity are multi-factorial, and it will be interesting to see if enhanced hepatic GH sensitivity, perhaps due to increased GHR density, also plays a role in the growth of obese children.*

Adda Grimberg, MD

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ASSESSMENT OF PSYCHOSOCIAL ASPECTS OF SHORT STATURE

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INTRODUCTION

The evidence is clear that growth hormone (GH) therapy can virtually eliminate the predicted height deficit for individuals with classic GH deficiency (GHD) if treatment is initiated at a sufficiently young age.[1] The unlimited availability of biosynthetic growth hormone (rhGH) has also made it possible to extend treatment to children who do not have GHD, but nonetheless exhibit short stature (SS) or poor growth. Consequently, the treatment of SS has become dissociated from its causes. Conditions for which rhGH is efficacious in promoting faster growth and taller stature include a diverse set of conditions: Turner syndrome,[2] chronic renal insufficiency,[3] Prader-Willi syndrome,[4] children born small for gestational age[5] and, most

Highlights In This Issue

E-Abstracts (Abstracts On-line)

From The Editor's Desk

Dear Colleague:

The increased number of abstracts and editorial comments published online has been very well received by readers of *GGH* journal. The feedback was praiseworthy, and there were a large number of viewers who accessed the e-abstracts. Both of these aspects are very rewarding to the Editorial Board. This issue also includes an expanded format; there are 8 abstracts published in the print version of the journal, plus one letter to the editor pertaining to the lead article dealing with pregnancy in T1DM patients (published in Volume 20, Number 4 of *GGH*). In addition, there are 6 papers published in the e-version, (accessed at www.GGHjournal.com). Altogether the Editorial Board canvassed and reviewed some of the most pertinent papers in the current literature. Finally, the lead article in this issue addresses a most important topic, one that pediatric endocrinologists deal with on a daily basis; namely, the evaluation of children with short stature. The paper by Sandberg and Colsman is an erudite review of the facts and pitfalls of the reports dealing with the psychosocial issues of short stature. They discuss the science and evidence and/or the lack of it, regarding the "heightism" prejudice that is so prevalent in our society. It constitutes an important contribution for those in practice dealing with short children, as well as for those interested in psychosocial research.

In May, 1985, I received an urgent call alerting me to the CJD association with the growth hormone that was used to treat hypopituitary patients. This hormone, extracted from cadaver pituitary glands, was immediately pulled off the market and we were left without any options to treat these patients. Fortunately, recombinant human growth hormone was in the pipeline and was soon available for clinical use. The 20th anniversary of this landmark accomplishment by Genentech is worthy of recognition.

Fima Lifshitz, MD

Growth, Genetics & Hormones is supported by an unrestricted educational grant from **Genentech, Inc.**

recently, idiopathic short stature (ISS), ie, short, but without diagnosable pathology.[6] In addition to eliciting improved growth velocity, rhGH has also been shown to produce metabolic benefits in particular conditions, eg, GHD, Prader-Willi syndrome, and chronic renal insufficiency.

The primary rationale for rhGH treatment has traditionally rested on the assumption that SS, in the extreme, may constitute a physical disability, and otherwise serves as a significant psychosocial burden for the individual. Furthermore, treatment is predicated on the belief that rhGH-induced increases in height will improve quality of life (QOL). Allen and Fost[7] infer from the growing number of conditions for which rhGH is prescribed that "the cause of short stature is not morally relevant in deciding who is entitled to treatment." These authors proposed that rhGH therapy is indicated when a "disability" in adaptation attributable to SS is identified (rather than by virtue of a medical diagnosis), and that treatment should be aimed at correcting this disability up to the point that an adult height within the "normal range" is attained, ie, 5th percentile.

This review summarizes what is known about the psychosocial aspects of SS and the QOL benefits of rhGH treatment. Stereotypes and assumptions about SS are evaluated in light of empirical findings. As described elsewhere,[8] studies and reviews were identified on MEDLINE® and PsychINFO® and The Cochrane Database of Systematic Reviews® using the terms "short," "stature," "height," or "growth hormone" combined with "psychological," "psychosocial," or "quality of life."

EVALUATING PSYCHOLOGICAL RESEARCH ON SS

Analogue versus "real world"

Research on stereotypical beliefs about those with SS is often conducted by assessing participants' perceptions in "analogue" studies. Social scientists employ analogue studies to answer well-defined research questions by isolating aspects of everyday life and assessing them within a controlled setting. The validity of findings stemming from such research designs has been questioned when used to investigate complicated social phenomena. Analogue studies of the psychosocial concomitants of SS that constrain information about the individual, or which place emphasis on stature, may unwittingly tap the stereotypes held by participants, but may be poor predictors of how participants perceive or treat an individual in the "real world."[9]

Descriptive cross-sectional studies

A common strategy is to use standardized questionnaires or interviews to assess psychological characteristics of individuals with SS, and then compare findings to those of individuals of average height. Such descriptive studies typically assess research participants at only one point in time and do not include an evaluation of an intervention, eg, response to a treatment such as rhGH. Validity of cross-sectional studies can be threatened by sample selection biases and participant reactivity.

a) Sample selection biases. Ascertainment of the psychosocial adaptation of individuals with SS depends on the composition of the targeted group. To evaluate the generalizability of the findings to all individuals with SS, research must provide details regarding the representativeness of the sample, ie, the proportion of those individuals eligible for study, based upon anthropometric criteria, who participate relative to those who do not. Factors resulting in an over-representation of better or poorer functioning individuals would bias the findings. Examples of clinic-based studies in which sample representativeness cannot be ascertained, and which report greater behavioral dysfunction among children and adolescents with SS, include 2 large studies.[10,11] Investigations that have more carefully recruited clinically representative samples of referred short youths have shown these groups to be similar in behavioral adjustment to population-based norms[12,13] and classmates.[14]

b) Comparison samples. The composition of comparison or control groups for individuals with SS is no less important than the selection of the target group when the goal is to make statements regarding the prevalence of problems. Factors contributing to recruiting a comparison sample that is functioning better than the general population would result in the SS group appearing less well-adapted.[15] Participant recruitment techniques which result in generally better functioning individuals include reliance on volunteers who are generally better adapted than those in the general population.[16] As an alternative to recruiting a control group, it is common to compare the target group (ie, youths with SS) with "norms" for the standardized method(s) administered. This practice is fraught with risks, including differences in inclusion and exclusion criteria and demographic characteristics that are related to participants' scores.[17]

c) Reactivity of assessment. An additional potential threat to the validity of a study stems from the subject's awareness of being studied.[9] The individual's motives and interpretations of the study can influence responses. For example, participants in clinic-based studies of the psychological adaptation of individuals with SS might assume that their role is to describe the liabilities associated with diminutive size, since they are being evaluated for short stature. If the participant's awareness of the assessment leads to a different response from usual, the measure is said to be *reactive*. Studies that have masked the examination of participant's height have failed to detect an association between height and psychosocial adaptation.[14,18]

d) Sources of information about the individual's psychosocial adaptation. Limited concordance in the reports of psychological adaptation across informants (child, parent, peers, others) is common and serves as a caution to readers of psychosocial literature regarding SS.[19] Stronger research designs involve the collection of data from multiple sources.[20] The most valid source of information about the social relationships of youths with SS would derive from studies utilizing peers as informants.[21] This strategy has been adopted in only 2 studies, one examining the social status of clinic-referred youth[14] and a community sample.[18]

Treatment studies

Studies that examine the influence of medical treatments (such as rhGH therapy) on psychological outcomes are vulnerable to threats stemming from evaluation bias introduced through either the informant's (often the parent) or examiner's knowledge that the patient is receiving the treatment, or placebo effects. In most research, the minimal experimental conditions include one group that receives an intervention and another group that does not (control group). The purpose of adopting a no-intervention group is to rule out alternative explanations for change in the intervention group, eg, placebo effects or regression toward the mean.

To the best of our knowledge, there has been only one clinical trial of the psychological effects of rhGH in children and adolescents that employed a randomized, placebo-controlled research design.[22] A recent meta-analysis suggests that placebo effects are stronger in clinical trials employing continuous *subjective* outcomes (such as measures of psychosocial adaptation) as compared to large trials employing dichotomous *objective* outcomes.[23]

The "regression toward the mean" should also be considered. This concept refers to the tendency of extreme scores on any measure to regress toward the mean of the distribution when the measure is re-administered. If individuals are selected for a study in a manner that they are more likely to generate extreme scores on a given measure, one can predict on statistical grounds that scores will tend to revert toward the mean on subsequent retesting.[15] To rule out this phenomenon as an explanation, changes observed in the treated group need to be compared with changes seen over the same time interval in a sample with similarly elevated baseline scores.[24]

Expectation biases may also be introduced into the data by relying on parent reports of children's behavioral adaptation. Parents' worries about their children's psychological adjustment to SS likely contribute to the referral to a pediatric endocrinologist and acceptance of a recommendation for rhGH therapy. These same worries may also be associated with an expectation (bias) that rhGH therapy results in reduced behavior problems. It is important to validate parental reports of psychological problems against other sources of information (eg, patients, teachers, or peers). Studies that have adopted this approach have demonstrated few differences between patients with SS and comparison or control groups.[12,18,20]

STATURE-RELATED STEREOTYPES

Stereotyping refers to a process in which identical characteristics are assigned to all individuals within a group, regardless of the actual variation among group members. Negative stereotypes regarding experiences and characteristics of individuals with SS are plentiful and categorized as: accompanying psychological characteristics, differential treatment by others, social relationships, and education/occupation (Table 1). Children's and adults' beliefs about height reliably demonstrate a bias toward the notion that "taller is better." With few exceptions, both children and adults attribute significantly less favorable characteristics to short individuals compared to those of tall or average height.[25-28] It is thus not surprising that youths and adults of both genders prefer to be taller.[29-31]

It has been suggested that individuals with SS experience disadvantages in the way they are treated due to stature-related societal perceptions.[32] As early as preschool age, mothers differentially treat girls based upon height.[33] Two studies in adults investigated the relationship between a person's height and "personal

Table 1. Empirical status of stature-related stereotypes

Stereotype	Evidence
Children and adults with SS are more poorly adjusted psychosocially	Generally supported by analogue (laboratory-based) research[25-28] Not supported by general population- or clinic-based studies[12,13,20]
Children and adults with SS are treated poorly due to their stature	Mixed results from analogue studies[33-35] Evidence of teasing and juvenilization from clinic-based studies[13,45]
Short men are less attractive and desirable to women as dates or husbands	Generally supported by analogue research[27,36,37] Limited support in population-based studies: effect attenuated when statistically controlling for confounding variables[53,58]
Children and adults with SS do less well at school/are less intelligent	Generally supported by analogue studies[33,40] Not supported by general population- or clinic-based studies of children[47-49] or adults[52,62]
Adults with SS hold lower status occupations and are paid less	Supported by analogue studies[27,41,42] Limited support in population-based studies: effect attenuated when statistically controlling for confounding variables[52-57]

space." Results were mixed: in one, the taller individual was afforded more space[34]; in the other, differences were not found.[35] Research on the effects of height on social relationships focuses on heterosexual dating and partner selection. For dating relationships, findings support the conventional notion that taller is more attractive, and this appears particularly true for males,[27,36-38] but less so for females.[27,38] Regarding the importance of height in partner selection, the man's height is more important a consideration for women than the reverse.[28,39]

When asked to evaluate classmates' competence, preschool boys rated small boys as better at "art" than tall boys; girls rated tall boys as smarter than small boys; but girls' height did not correlate with ratings.[33] Mothers rated tall boys and girls as more competent than small boys in the majority of domains,[33] and had greater expectations for mastery and achievement from taller children.[40] With regard to adults' occupational status, undergraduates judged individuals who have more prestigious occupations as taller than those of less prestige.[41,42] They also expected taller people to have a higher professional status than shorter people.[27]

QOL ASSUMPTIONS REGARDING SS

Assumption 1: Patients with SS experience chronic psychosocial stress (Table 2). Early studies showed that SS is associated with teasing and juvenilization.[43] These investigations were generally restricted to patients with complex medical conditions with little attention directed toward bias introduced by subject selection factors.[44] Two relatively recent clinic-based studies found that approximately 60% to 70% of patients referred to pediatric endocrinologists for a growth evaluation had experienced teasing or juvenilization, and that these stressors were experienced with some regularity.[13,45] Contrary to expectations, however, the child's relative height (–3.1 to –0.2 height SD) was not significantly related to the incidence of these negative experiences.[13] Furthermore, the presence of psychosocial stress does not imply that SS constitutes a "disability".[7] To rise to this threshold, it would be necessary to provide clear evidence that these stressors are associated with clinically significant impairment in social, academic, or occupational functioning.

Assumption 2: Patients with SS exhibit clinically significant problems of psychosocial adaptation. It is commonly believed that patients with SS exhibit clinically significant behavioral or emotional problems.[43] Implicit in this assumption is the expectation that psychiatric problems are significantly more common among patients with SS than in the general population (rates of childhood psychiatric disorders fall between 18% and 22%).[46] However, this does not appear to be the case when selection biases in participant recruitment are minimized. For example, self-esteem scale scores for short youths referred for evaluation of SS were higher (ie, more positive) than questionnaire norms, despite reports that the majority of these individuals experienced teasing and juvenilization.[13] The same was true for behavior disturbance: patients reported significantly fewer problems than questionnaire norms, and parental reports indicated that patients were indistinguishable from the norms in behavioral and emotional functioning.[13] Similar findings were reported in other clinic-based studies.[12,20] In contrast, other studies report significantly more behavioral and emotional problems among children with SS relative to norms as measured by self- and parental-report.[10,11] Unfortunately, key details essential to gauge the representativeness of these samples[10,11] were not provided, such as the total number of eligible patients and the method of targeting participants for behavioral studies.[24] Studies featuring clinically representative samples show behavioral adjustment is comparable to classmates[14] and to population-based norms.[12,13]

Table 2. Assumptions underlying growth-promoting therapies

Assumption	Evidence
Patients with short stature experience chronic psychosocial stress	Supported by clinic-based studies[13,45]
Patients with short stature exhibit clinically significant problems of psychosocial adaptation	Not generally supported[10,12,13,20,22]
Short youths and adults in the general population are similarly at risk for problems of social adjustment	Not supported in children, adolescents[18,48,50,51] or adults[52,56,58]
Stature-related social stress results in significant problems of psychosocial adjustment	Limited support: though teasing and juvenilization were related to behavior problems,[45] overall psychosocial adaptation was equivalent to community norms[12]
Increases in growth velocity and height induced by rhGH therapy result in an improved QOL	Not supported[20,22,31,62]

Corollary of Assumptions 1 and 2: Individuals with SS in the general population also exhibit significant problems of psychosocial adaptation. Although rarely articulated, it follows from both preceding assumptions that short youths who are *not* referred for a medical evaluation are similarly at risk for psychosocial adaptation problems. In the prospective, longitudinal Wessex Growth Study, in which the sample comprised short, healthy children from the general population, no evidence of serious psychosocial or academic disadvantage was found.[47-50] Although individuals in the SS group preferred to be taller, and reported more bullying than their taller peers,[29] neither the desire for physical change nor bullying

had measurable effects on school performance or self-esteem,[47,48,50] suggesting that stigmatized individuals use self-protective cognitive mechanisms that allow self-esteem to remain intact.[12]

In the largest study of its type, and the only one conducted on a national probability sample of the U.S. population, Wilson and colleagues[51] assessed the relationship between stature, IQ, and academic achievement. Statistically controlling for potentially confounding background characteristics, subjects' height contributed significantly (approximately 2%) to the prediction of both indices. The Wessex Growth Study replicated this general finding. However, as in the U.S. study, height explained only 2% of the variance in IQ. Socioeconomic factors, rather than stature, best predicted psychosocial and academic outcomes.[48]

In a recent study using a novel research design, the influence of height on students' (N=956, grades 6 –12; approximately 11–18 years old) psychosocial adaptation was assessed using peer informants.[18] Statistically significant relationships were not detected between height and measures of friendship, popularity, or most aspects of reputation among peers, despite substantial statistical power. Findings did not vary by participant gender, peer- or self-report, whether data from the entire sample were used, or when subgroups of very short (≤ –2.25 height SD; 1st percentile) or very tall students (≥ 2.25 height SD; 99th percentile) students were contrasted with average height (25th–75th percentile) classmates. In the lower grades, classmates perceived shorter students as younger than their age. However, this perception was not meaningfully related to measures of social acceptance or other aspects of reputation among peers. The authors concluded that extremes of stature in the general population—either short or tall—have minimal detectable influence on peer perceptions of social behavior, friendship, or acceptance.[18]

A statistically significant relationship between men's heights and the likelihood of completing college was not found.[52] Taller men were not more likely to achieve higher professional status when analyses controlled for educational attainment.[52] Studies of the relationship between height and income often report that tall men and women earn more than their shorter colleagues.[52-56] However, when potentially confounding variables such as age, health, education, and family of origin characteristics are controlled for statistically, the relationship between height and income is attenuated.[52,56] In a cohort study of all healthy Swedish military conscripts in 1994, short conscripts (< –2 height SD) exhibited more physical and mental health problems and scored lower on tests of intellectual performance than taller men.[57] The investigators raised the possibility that the association between height and physical and psychological adaptiveness are indirectly linked. For example, biological factors that contribute to poorer growth may also be responsible for poorer physical performance and more limited intellectual aptitude.

The relationship between height and marriage rates varies by study. In the National Child Development Study (a longitudinal study of British citizens), the probability of being married was 7% lower for short men (<9th percentile) and 5% lower for tall women (>90th percentile) than for adults of average height (20th–79th percentiles), when statistically controlling for social class, education, health, race, and region of residence.[53] Contrasting findings were derived from the U.S. National Longitudinal Survey of Youth, a study featuring a comparable research design. Although short men exhibited lower rates of first marriage than those of average height, this effect disappeared once family-of-origin variables (parental education, poverty status, and region of the country) were taken into account; no consistent relationship was found between women's height and marriage rates.[58,59]

Assumption 3: Height-related social stress results in significant problems of psychological adjustment. As both teasing[60,61] and psychological adaptation problems[46] are relatively common among children and adolescents, support for Assumption 3 should come from a demonstrated statistical link between stressful stature-related experiences and psychosocial dysfunction. In the only study that specifically addressed this issue, parental report of stature-related teasing significantly predicted increased emotional problems.[45] The proportion of unique variance in problem scores attributable to teasing was approximately 2% and increased (to between 4% and 5%) when the frequency of teasing was taken into account. Juvenilization also contributed unique explanatory value, and summated with teasing as a negative influence on psychosocial adaptation.

To interpret the clinical significance of these effects, one must view them within the context of the mean level of behavior problems in this sample. As noted earlier, the psychological adaptation of short youths in this same clinic-referred cohort was comparable to community norms.[12] Thus, the possibility exists that stature-related stresses may contribute to variability in adaptation that falls within the "normal range."

Assumption 4: Increased growth velocity and height induced by rhGH therapy result in improved QOL. There are very few randomized, controlled trials of the QOL benefits of rhGH treatment (and only one randomized placebo-controlled trial[22]). In the Wessex Growth Study rhGH-treated children with ISS were compared with those in an untreated control group at recruitment and after 3 and 5 years.[62] Despite a significant increase in

height in the treatment group, there were no differences between the groups on the behavioral measures at any of the 3 assessments. Comparable results were found in a more recent study in which, despite increased height in the treated group, no improvement on self- or parental-report measures of psychosocial adaptation and self-esteem were found.[20] In a recently published report on the psychological benefits of rhGH therapy, youths with ISS were randomly assigned to either treatment or a control group which received placebo injections.[22] At baseline, the behavioral/emotional adjustment and self-esteem scores for children with ISS were within the normative range. Furthermore, no systematic relationship was observed between attained height SDs, or the change in height SDs from baseline and annual changes in behavior problem or self-esteem scores. Finally, in a retrospective study of young adults who either had or had not been treated with rhGH therapy for ISS, no differences in education level or QOL were found,[31] though the treated patients had a romantic partner less often than participants who did not receive rhGH therapy in childhood.

Although the focus of the "treat or not to treat" debate is directed at rhGH therapy, androgen treatment of boys with constitutional growth delay has long been a strategy to accelerate growth velocity, hasten the onset of secondary sex characteristics and, thereby, ameliorate perceived psychological distress, without sacrificing adult height.[63-65] There has never been a comparison of the psychological benefits of rhGH versus androgen therapy. A direct comparison is tantalizing considering the differences in the objective of treatment (ie, hastening pubertal progression versus achievement of taller adult height), duration of treatment, and cost.

RECOMMENDATIONS

Practice guidelines for the use of rhGH therapy in children with SS state that decisions regarding "instituting or continuing therapy should be individualized...and be guided by the goal of improving the quality of life of the child and future adult."[66] These recommendations are echoed by Allen and Fost[7] who emphasize that access to rhGH therapy should be guided by the identification and amelioration of disability stemming from SS. Identifying those who experience SS as a "disability" is a challenging task. The fact that the child or adolescent experiences teasing or juvenilization, or that the family is seeking a consultation from a pediatric endocrinologist, are insufficient reasons to make this determination. Psychosocial stress is a common phenomenon in child development and, by itself, does not imply psychiatric dysfunction or even significant problems of psychosocial adaptation. Noeker and Haverkamp[67] developed a useful conceptual framework to guide the psychological assessment of SS which can be used to inform clinical management decisions. Three hierarchical levels of assessment are identified: stress exposure due to SS (Level I), quality of coping responses (Level II), and occurrence of psychopathology (Level III) (Table 3).

Table 3. Psychological assessment of the short child (adapted from reference 67)

Target of Assessment	Information Collected
Level I	
• Stress associated with condition	• Stigmatization and juvenilization associated with SS • Other stressors associated with the medical syndrome – Experiences of stress (Level I) do not imply psychiatric dysfunction (Level III)
Level II	
• Quality of adaptive coping responses	• Behavioral and emotional propensities in response to stresses • Individual and family characteristics serving to attenuate or amplify maladaptive responses to stress (ie, risk and protective factors)
Level III	
• Occurrence of behavioral or emotional adaptation problems • Impairment in family, peer, or educational functioning	• Range and intensity of problems and their coalescence into psychiatric syndromes • Presence of "impairment" in key psychological development domain: family, peer, or educational functioning – "Dissatisfaction" with height does not imply impairment in function

Clinical management is facilitated by a thorough psychosocial evaluation designed to delineate specific stressors experienced by the child, the pattern of coping, and psychosocial adaptation. Because of the salience of SS and its potential to serve as a lightning rod diverting attention from other stressors, clinicians must be watchful of misattributions by the child, parents, or others (including oneself[20]). This influence may direct attention away from prescribing psychosocial interventions for maladaptive coping.[68] This evaluation serves to assess individual characteristics (eg, intelligence, temperament) and social-ecologic factors (eg, degree of stress in the child's environment, salience of height to the family, social support from peers) that could moderate the influence of height on psychosocial adaptation. Finally, identifying adaptive coping strategies as an

alternative (or adjunct) to rhGH therapy is an additional goal. Gathering such detailed information is prudent in view of the clinical evidence showing that the adult height of formerly treated GH-sufficient individuals often remains substantially below average.[6,69-71]

The comprehensive nature of this evaluation implies that it should be conducted by a mental health professional—ideally by a member of the pediatric endocrinology team, knowledgeable in both the medical and psychosocial aspects of SS. The team member is in a position to delineate predictable psychosocial experiences related to SS and to offer anticipatory guidance to patients and families. The entire team should reassure parents that SS does not have to limit their child's current or future happiness, success, or productivity. However this is an ideal model that most often is not applied in clinical practice, even in most academic centers. Thus, the practicing physician caring for children with SS needs to balance the "do's and don'ts" (Table 4) before casting assumptions for the consequences of SS and the recommendations for treatment.

Parents may evaluate factors for and against rhGH therapy differently from physicians.[72,73] Factors parents consider (in order of descending importance) include risk of long-term side-effects, out-of-pocket costs, the child's attitude toward wanting rhGH therapy, the likelihood of a height increase, the magnitude of the height increase, and the route of rhGH administration.[72] Given the importance of these to families, it is prudent to gear interactions toward addressing these priorities. To this list, we would add the importance of making explicit the assumptions that the child, family, and physician hold concerning the liabilities of SS and the expected benefits of rhGH therapy (Table 4).

Table 4. Recommendations for clinicians

Do's	Don'ts
Conduct a comprehensive psychosocial assessment[44,67]	If problems of psychosocial adaptation are detected, do not assume that these are attributable to SS
Recommend psychosocial strategies to directly address predictable social challenges associated with SS[68]	Do not neglect the psychosocial implications of features other than SS associated with particular syndrome
Balance medical recommendations with suggestions to address any psychosocial stress associated with SS[67]	Do not assume the parent or patient wants rhGH therapy
Discourage the expectation that taller stature is associated with changes in QOL[18,20,31,62]	Do not restrict discussion of side effects (known and unknown) while emphasizing safety
Be aware of and address factors the parent and patient use in making their decision[72,73]	Do not minimize potential monetary costs of rhGH therapy; discuss these prior to initiating therapy
Discuss treatment efficacy in terms of the degree of certainty and magnitude of effects[6]	

CONCLUSION AND SPECULATION

Commonly held beliefs and attitudes serve as implicit assumptions in the QOL rationale for applications of rhGH therapy beyond the traditional role of hormone replacement. In view of the findings on stereotypes, particularly research findings gleaned from laboratory studies, it is understandable that parents of children with SS may be concerned about their child's psychosocial and educational adaptation. However, findings from clinic- and general population-based research on the real-world experiences of youths and adults with SS do not generally support the view that SS is associated with psychological dysfunction, ie, constitutes a "disability".[7] Similarly, research on the QOL benefits of rhGH therapy does not demonstrate efficacy for this outcome.

What might account for the stability of negative stereotypes and assumptions regarding SS despite contradictory evidence? Schkade and Kahneman[74] proposed that a "focusing illusion" potentially accounts for such a phenomenon. Assuming (with considerable evidence to support it[8,26]) most believe that SS is associated with multiple negative characteristics, it follows that evaluations of an individual's QOL that focus on this isolated trait would be overly negative. The focusing illusion occurs "when a judgment about an entire object or category is made with attention focused on a subset of that category, . . . whereby the attended subset is overweighted relative to the unattended subset."[74] Schwarz and colleagues (as cited in [74]) described one instance of the focusing illusion. In their study, college students were asked 2 questions: "How happy are you?" "How many dates did you have last month?" The correlation between responses to the questions depended on which question was asked first. When the happiness question came first, the correlation was 0.12. However, when the dating question preceded the one on happiness, the correlation rose to 0.66. Thus, focusing on one aspect of life to the exclusion of others results in overweighting of that factor in the experience of well-being. In the case of the individual with SS who is being queried about social experiences they believe are linked to height, the context of questioning encourages the respondent to focus on this one aspect of their life to the exclusion of others. Under these circumstances, responses are likely to be overweighted in the negative direction because of the shift of focus away from compensating factors. The focusing illusion thus serves as a potential explanation for why our perceptions of the QOL of others—in this case those with SS—seems to be off the mark. The existence of a focusing illusion may also serve as a cautionary

note for parents and clinicians. The possibility exists that by focusing on height, this characteristic becomes overvalued relative to less salient ones. Ironically, the treatment with rhGH of individuals who are destined to be shorter than average, and the attendant focusing of attention and energy over years, may potentially amplify the negative influence of this cognitive phenomenon.

In conclusion, the data summarized indicate that most individuals with SS adapt psychologically to the common psychosocial stresses associated with height. These positive findings notwithstanding, family and physician concerns for the child may be influenced by prevalent stature-related stereotypes and prejudices. Furthermore, the conclusion that individuals generally make positive adaptations to difficult circumstances should not be used as a justification to ignore stresses that may be remediable. It is worth remembering that subgroups of children (and households) are already facing multiple challenges to healthy psychological function, and that the burden of teasing or juvenilization may push the balance from adaptive to maladaptive coping. Valid "remedies" for children experiencing stress (and distress) related to SS will likely come about through individualized treatments involving both psychosocial and medical interventions, including the use of growth-promoting medications.

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ABSTRACTS FROM THE LITERATURE

Islet Cell Transplantation in T1DM

Islet cell transplantation has succeeded in restoring insulin independence in type 1 diabetes (T1DM) patients. However, islet allografts from 2 to 4 donors have been required to transplant an appropriate cell mass. This paper described the safety and efficacy of a single-donor, marginal-dose islet transplant protocol in 8 women with T1DM, nocturnal hypoglycemia, and advanced secondary complications. Each patient received a small dose of islet cell allotransplants from a single cadaver donor pancreas after antithymocyte globulin, daclizumab, and etanercept, and were immunosuppressed with mycophenolate mofetil, sirolimus, and no- or low-dose tacrolimus. All 8 patients achieved insulin independence and freedom from hypoglycemia; 5 remained insulin-independent for longer than 1 year. Graft failure occurred in 3 patients preceded by sub-therapeutic sirolimus trough levels (<9 ng/mL) in the absence of tacrolimus trough levels (<9 ng/mL). The authors concluded that improved islet cell engraftment was secondary to the peritransplant administration of antithymocyte globulin and etanercept.

Hering B J, Kanadaswamy R, Ansite JD, et al. Single-donor, marginal-dose islet transplantation in patients with type 1 diabetes. *JAMA*. 2005; 293:830-835.

Editor's Comment: *Transplanting insulin producing cells from fresh cadavers into T1DM patients is known to reverse the disease, but the procedure has been too costly and fraught with difficulties for widespread use. The authors of this study showed that their protocol was effective, safe, and less costly, as a single donor cadaver was sufficient to produce an appropriate dose of islet cells for transplantation. These allografts took residence in the liver of the patients and started producing insulin. Although 3 patients rejected the transplant, they achieved insulin-independence and freedom from hypoglycemia for 127, 76, and 7 days. In previous trials there was a need to utilize 2 to 4 cadavers, and each infusion of cells cost about $75 000, including follow-up treatments. In the new trial there was a cost saving, since only one pancreas was needed and there was a need for less diabetogenic immunosuppressants. These findings are of interest and may have implications for a not very distant day when this type of therapy will be routine in clinical care of T1DM patients.*

Fima Lifshitz, MD

Growth Hormone Receptor: In Vivo Analysis of the Cytoplasmic Signaling Domains

In vitro studies of the growth hormone receptor (GHR) have identified multiple post-receptor signaling pathways including JAK2 tyrosine kinase, STAT5, ERK1/2, PI3-kinase, a JAK2-independent calcium signaling element, SHP2 phosphatase, SOCS and CIS. Although STAT5 is primarily responsible for GH-induced expression of insulin-like growth factor (IGF)-I, STAT5b$^{-/-}$ mice have less severe growth retardation than GHR$^{-/-}$ mice, indicating a physiologic significance of alternative pathways.

Rowland and colleagues undertook the impressive task of teasing apart the GHR signaling domains *in vivo*. They created 2 knockin mice bearing truncated GHR mutants: m569 was truncated at residue 569 (wild-type GHR contains 650 amino acids) and had site-directed mutations of tyrosines 539 and 545 in order to delete 70% of the STAT5 docking sites, while m391 was truncated at residue 391, thereby also deleting the proximal STAT5 sites (0% STAT5 signaling left) while retaining 100%

LETTER TO THE EDITOR: PREGNANCY IN T1DM ADOLESCENTS

Thank-you for your comprehensive article that highlighted the potential complications of pregnancy in teenagers with type 1 diabetes (T1DM). We were surprised at your findings that "chronically ill adolescents are less likely to receive contraceptive counseling and sexual education than their healthy counterparts".[1] The clinical practice guidelines in Canada and the United States clearly require that adolescents with T1DM receive counseling on contraception and sexual health to avoid unplanned pregnancy.[2,3] Given that adolescents with T1DM see a physician 3 to 4 times per year, there is much more opportunity to discuss sexual health and birth control issues compared to adolescents without chronic disease.

We are a multi-disciplinary team in a pediatric diabetes education and care program for children with diabetes. Pediatric endocrinologists, a masters-prepared social worker, nurses, and dietitians make up our team. As a quality assurance activity, we surveyed adolescents age 12 to 18 years with diabetes in our program from January to August 2001 to determine rates of smoking and sexual activity and their recall of teaching on these subjects.[4] We found 11.8% of adolescents with T1DM were sexually active compared to the Canadian national average of 44% for females age 15 to 19 years.[5] Only 5.9% of our adolescent males with T1DM reported sexual activity compared to 43% of Canadian boys age 15-19 years.[5] Sixty percent of adolescent girls and 40% of adolescent boys in our clinic reported having being involved in a discussion about sexuality in the previous year. We understand that our rates may be low due to reluctance of the patients to divulge this information and because we included younger adolescents in the survey.

Our team begins education about adolescent issues including sexuality, birth control, and preconception counseling at approximately age 12 years. Oral contraceptives are encouraged as well as barrier methods to prevent sexually transmitted diseases. We know of only one pregnancy in more than 500 females with T1DM in our program since 1985. Our concern lies in the difficulties that adolescents face when they are transferred to adult care at age 18.[6] The rate of dropout of diabetes care in young adult years has been found to be 25%,[7] and this is alarming considering the risk of pregnancy without early care, as you clearly state in your paper. Our focus needs to be on supporting young adults through the stress of transition while remaining ever vigilant in the care of our adolescents in preventing pregnancy.

Sincerely,
Gillian Toth, RN, CDE; Heather Dean, MD, FRCPC; Elizabeth Sellers, MD, FRCPC; Janet Grabowski, MD, FRCPC; Louise Rawluk, RN, CDE; Nicole Aylward, RD, CDE; Norma VanWalleghem, RD, CDE; Gen Henderson, MSW, CDE; Catherine MacDonald, BFA(H)
Diabetes Education Resource for Children & Adolescents
Department of Child Health
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Author's Response

We appreciate your letter and kind words concerning our review on pregnancy in adolescents with T1DM, and we are grateful to learn of your comprehensive care program for adolescents with diabetes.[4] Such programs are ideal and hopefully will translate into a decrease in the rate of pregnancies in diabetic adolescents. You are correct in quoting the guidelines in Canada and the United States that mandate education on contraceptive counseling and sexual education.[2,3] Unfortunately, these guidelines are not a guarantee for appropriate contraception. The quality assurance activity that you reported[4] may not reflect the true prevalence of sexual activity among T1DM adolescents. Your comparison group of non-diabetic adolescents (1994–95 National Population Health Survey) reported an estimated 43% of girls aged 15 to 19 years had at least one sex partner in the previous year. In addition, among sexually active 15- to 19-year-old adolescents, 51% reported having sex without a condom in the past year and less than 50% of the adolescent females who admitted to sexual activity reported using oral contraceptives.[5] These surveys are not available for comparison in T1DM.

You reported that 69% of adolescent females in your clinic have been involved in a discussion about sexuality in the last year. The impact of these discussions to lower the pregnancy rate in T1DM patients is yet to be shown. Intensification of methods that prevent pregnancy in this high-risk population need to be implemented. Offering effective contraception, even without consent of the parents, may be the only means to decrease the pregnancy rate in adolescents. Hopefully, your program will provide the evidence and strategies that are applicable to diabetic adolescents and thus spur increased efforts to focus on pregnancy prevention.

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of JAK2 and ERK1/2 signaling. Radioreceptor assays confirmed normal levels of specific binding of ^{125}I-labeled GH, expressed per milligram of membrane protein, in the 2 mutant mice. Comparison to wild-type mice showed 44% of GH-dependent growth in the m569 mice and 11% growth in the m391 mice. Serum IGF-I levels were 16% to 21% of wild-type in m569 and less than 10% in m391. However, hepatic IGF-I transcript levels were not depressed as much, suggesting additional IGF-I protein clearance due to decreased ternary complex formation from reductions in IGFBP-3 and ALS expression. Both mutants developed obesity in males after 4 months of age, as well as associated hyperglycemia.

The authors took their characterizations one step further: microarray analysis of the 2 mutant mice compared to wild-type and GHR$^{-/-}$ mice revealed domain-specific regulation of different target genes. Four hundred three transcripts (398 genes) were differentially expressed across all groups, 20 were common to all, 13 unique to m569, 59 unique to m391 and 268 unique to GHR$^{-/-}$. Interestingly, only 5 genes were regulated exclusively by residues 569-650; thus the distal 70% STAT5 binding played a minor role in mediating the genomic effects of GH. IGF-I was one of 20 STAT5-regulated genes, and the proximal 30% STAT5 binding was important for inducing IGF-I. The majority of regulated transcripts related to the more proximal GHR domains, where JAK2 leads to ERK1/2 and PI3-kinase signaling and SOCS proteins play an inhibitory role. These included many metabolic genes and genes related to hepatocyte function such as signaling, proliferation, translation, and transporter proteins. I refer the reader to the paper and the associated website (http://research.imb.uq.edu.au/~mwaters/ghr/) for detailed listings.

Rowland JE, Lichanska AM, Kerr LM, et al. In vivo analysis of growth hormone receptor signaling domains and their associated transcripts. *Molec Cell Biol.* 2005; 25: 66-77.

Editor's Comment: *This tremendous piece of work significantly advances our knowledge of GHR function; not only is GHR signaling dissected to a sharper degree than before, but new GH functions are suggested by the target genes identified in the microarray analyses. How does all this correlate clinically? GHR mutations cause GH-insensitivity syndrome, or Laron syndrome, characterized by severe postnatal growth retardation, low circulating IGF-I levels despite elevated GH levels, and lack of IGF-I response to rhGH. The majority of reported mutations occur in the extracellular part of the protein; defects in the cytoplasmic domains of GHR, studied in this paper, are rare. However, 2 recent papers described patients with distal cytoplasmic GHR mutations resulting in selective loss of STAT5 pathway. Two siblings, in their 50's, had homozygous deletions that encoded GHRs truncated at amino acid 449; loss of STAT5 binding, despite retention of normal JAK2 phosphorylation, STAT3 and ERK2, was sufficient to cause severe growth failure (height z scores of –8.7 and –6).*[1] *A 17-year-old girl was identified with a height z score of –5.28 and classic features of Laron syndrome. She was a compound heterozygote for novel GHR mutations: C83X (lack of GHR expression due to mRNA decay or defect in cell membrane anchoring) and 1776del (GHR truncated at 581 amino acids). The 1776del GHR had significant impairment of STAT5 activation despite intact extracellular, transmembrane and more than 80% of the cytoplasmic GHR domains. STAT3 activation was normal.*[2] *The clinical importance of STAT5 signaling was further confirmed in a 16.5-year-old girl whose Laron syndrome was caused not by a GHR mutation, but by a homozygous missense mutation in the STAT5b gene; her height z score*[3] *was –7.5.*

Adda Grimberg, MD

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Anthropometry, Metabolic Control, and Thyroid Autoimmunity in Type 1 Diabetes with Celiac Disease: A Multicenter Survey

The DPV-Wiss database is a central registry for the documentation of treatment processes and outcomes in children with type 1 diabetes (T1DM) in Germany and Austria. Data are gathered and available for analysis from 150 pediatric departments and 19 796 subjects (ages 0.1 to 19.9), representing approximately half the children with T1DM in Germany. Kaspers et al report their findings regarding the association between T1DM and celiac disease (CD) and anthropometrics and metabolic control. Although there is no consensus for measuring celiac and thyroid antibodies in children with diabetes, in Germany these determinations are commonly performed every 1 to 2 years.

Three groups of diabetic children were identified: Group 1 comprised no clinical or biochemical signs of CD (n=18 470); Group 2 had 1 or more significantly elevated CD-associated antibodies (IgG and IgA antibodies to gliadin, endomysium, or tissue transglutaminase–IgA antibodies), but no jejunal biopsy performed or recorded (n = 1 119); Group 3 exhibited biopsy-proven CD (n = 127). At least one CD associated antibody elevation was present in 6.7% of the cohort, while 0.6% had histologically confirmed CD. The mean age of diagnosis of CD was lower than that of the Group 1 subjects (12.2 ± 4.6 years vs 13.4 ± 4.3 years,

$P< 0.05$) and the onset of T1DM was at an earlier age in Group 3 children (5.8 ± 4.0 vs 8.2 ± 4.0 years, $P<0.001$). The average age of diagnosis of CD was 4.3 ± 3.8 years. In 13 children, CD was diagnosed prior to the diagnosis of T1DM; 57% of Group 3 children were female.

Standing height was significantly reduced in Group 3 children (–49 ± 1.1 vs –0.06 ± 1.0 height-SDS, $P<0.001$), and this difference increased over time. Growth of children less than 11 years of age was more affected than that of older children. BMI was also lower in the CD children and did not improve over time. Daily insulin requirements, number of daily injections, and number of severe hypoglycemic episodes did not differ among groups, but HbA1c levels were significantly lower in the Group 3 children at CD diagnosis (8.1% ±1.8% vs 8.8% ± 2.4%, $P<0.001$) and remained lower during the observation period. The incidence of thyroid disease was greater in the group with T1DM and CD (6.3% vs 2.7%, $P<0.02$).

The authors noted that the actual incidence of CD in their population may be greater than recorded, since all children with positive antibodies did not undergo jejunal biopsy. The failure of children with CD to exhibit catch-up growth may have been the result of poor compliance with the gluten-free diet. The growth reductions seen in these children would account for a loss of 5.2 cm in final height. The lower HbA1c values in the Group 3 children could have been the result of malabsorption of nutrients or perhaps more meticulous attention to carbohydrate amounts and source when using the gluten-free diet. The authors concluded that the recommendations for regular screening of children with T1DM for CD beginning at disease onset is both rational and important, as untreated CD is associated with significant long-term health risks such as osteoporosis and intestinal lymphoma.

Kaspers S, Kordonouri O, Schober E, Grabert M, Hauffa B, Holl R and the German Working Group for Pediatric Diabetology. Anthropometry, metabolic control, and thyroid autoimmunity in type 1 diabetes with celiac disease: A multicenter survey. *J Pediatr.* 2004:145:790-795.

First Editor's Comment: *This paper presents important data confirming the relationship between CD and T1DM; it lends evidence for the need to screen children with T1DM for celiac associated antibodies at the time of diagnosis and throughout childhood. The findings of reduced height and BMI, not related to poor glycemic control, are of concern. The implementation of a gluten-free diet is difficult and compliance is even more difficult to assess than that of the more routine carbohydrate-counting caloric recommendation diet usually prescribed for children with T1DM. It will be important for studies such as the current one to continue in order to assess the long-term impact of living with both diseases. Information regarding the development of osteoporosis and lymphoma in asymptomatic children is especially important. The current database may assist in the collection of such information.*

Currently, some third-party payors in the United States are reluctant to reimburse for the laboratory determinations of celiac associated antibodies in children with T1DM who do not exhibit typical symptoms and signs of CD. The data from this German database can be used to help justify such screenings.

William L. Clarke, MD

Second Editor's Comment: *It is important to distinguish CD from celiac autoimmunity (CA). The former presents with symptoms and abnormalities on the intestinal mucosa and function which improve with a gluten-free diet; the later presents no alterations other than positive IgA transglutaminase antibodies and enodmysial antibody immunofluorescence IgA. The natural history of CD is well known, including chronic intestinal malabsorption and long-term risks of osteoporosis and lymphoma. However, no such data exist for CA patients who have no symptoms or alterations in jejunal morphology and function; however, monitoring height and weight progression is warranted. Since adherence to a gluten-free diet is poor even in the CD patients, the added burden to diabetic individuals would need to be considered before recommending it when CA is encountered. The reader is referred to GGH[1] for a review and commentary of a paper by Hofferberg[2] on the natural history of CA.*

Fima Lifshitz, MD

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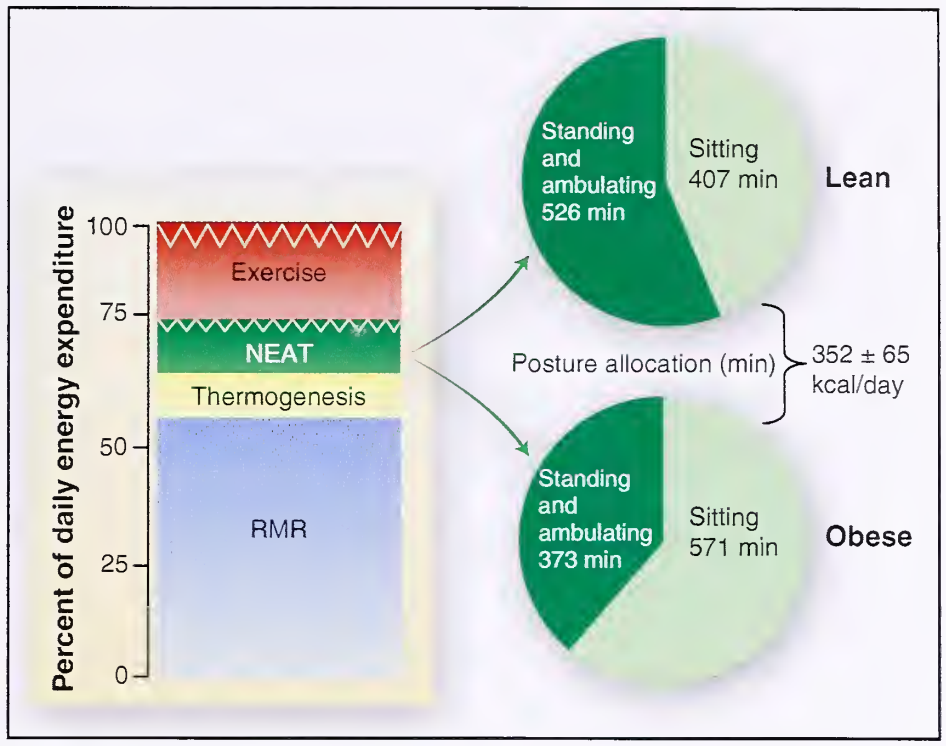
Movement and Energy Expenditure in Obesity

The authors quantitated the movement and energy expenditure of 20 healthy self-proclaimed "coach potatoes." Ten lean (BMI = 23 ± 2 kg/m^2) and 10 modestly obese (BMI = 33 ± 2 kg/m^2) volunteers donned and wore a physical activity monitoring system (PAMS) for 10 days while continuing their normal occupations, hobbies, and day-time and night-time activities, and while consuming a diet designed to maintain a constant body weight. Energy expenditure related to purposeful exercise and that related to routine activities of daily living, non-exercise activity thermogenesis (NEAT), was determined. NEAT was further divided into energy expended in relation to posture (lying, sitting, standing) and energy utilized for movement (ambulation). The findings revealed that both groups slept (lying) for similar intervals, but that obese subjects sat 164 minutes per day longer and stood 152 minutes less per day than did control volunteers (Figure). This translated into a mean lower daily energy expenditure

of 352 kcal. Neither supervised weight loss (8 kg) in 7 obese subjects nor weight gain (4 kg) in 10 lean volunteers altered the distribution times of posture and movement, suggesting that these activities were "intrinsic" to the individual rather than environmentally determined.[1] However, the mechanism(s) that regulates posture and movement distribution are not known. The investigators suggest that if an obese subject were to increase daily caloric expenditure by 350 kcal (without corresponding increase in calorie intake, of course), over the course of 1 year there would be a 15 kg weight loss!

Levine JA, Lanningham-Foster LM, McCrady SK, et al. Interindividual variation in posture allocation: Possible role in human obesity. *Science*. 2005;307:584-586.



The components of daily energy expenditure are depicted (left) and the differences between lean and obese subjects are shown (right). The pie charts show the cost of unplanned physical activity (NEAT).

Editor's Comment: *It has long been known by clinical observation that very obese subjects move imperceptibly when sitting (ie, they do not fidget) and choose to sit when others in the vicinity are standing; by inference they must be conserving every calorie. However, present data provide quantitative proof on this propensity even in only modestly obese individuals. The method for measurement of PAMS was designed by Levine and consisted of 6 sets of sensors embedded in special underwear, 4 "inclinometers" attached to the trunk and thighs, and 2 "triaxial accelerometers" fixed to the base of the spine.[2] Each subject wore this unit 23:45 hours daily (15 minutes for showering) for 10 days. These instruments recorded data every half-second providing information on body position and motion 1 728 000 times over 10 days per subject! Experimentally, injection of orexin into the paraventricular nucleus of rats increases NEAT, implying that posture and movement may be modulated by neural transmitters. Clearly, efforts to increase NEAT in our obese patients are worthwhile—primarily by substituting physical activity such as walking for television viewing and game playing.*

Allen W. Root, MD

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Growth, Genetics & Hormones

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The Many Faces of *PTHR1* Mutations

Gain-of-function mutations of the gene encoding the parathyroid hormone (PTH)/PTH-related peptide (PTHrP) type 1 receptor (*PTHR1*) gene cause the severe, dominantly inherited metaphyseal chondrodysplasia, type Jansen. Loss-of function mutations of this receptor are associated with osteosclerosis and advanced skeletal maturation of the recessively inherited Blomstrand chondrodysplasia. Eiken syndrome is a rare autosomal recessive bone dysplasia with a skeletal phenotype quite different from the other 2 conditions, most notably exhibiting multiple epiphyseal dysplasia with extremely delayed ossification.

Duchatelet et al mapped Eiken syndrome in an informative family to the region *PTHR1* locus. Mutation analysis revealed an ARG485STOP nonsense mutation in the last exon that predicts truncation of the last 108 amino acids from the receptor's cytoplasmic tail. This domain contains several elements critical to the function of the receptor. These include several serine residues that are phosphorylated upon ligand binding and docking sites for proteins that propagate PTHR1 signals including G-protein receptor kinases (ie, adenyl cyclase(AC)/protein kinase A (PKA), phospholipase C (PLC), protein kinase C (PKC)) and ß-arrestin as well as residues that participate in the receptor internalization and down regulation.

The authors did not carry out functional studies, but they speculated based on what has been previously reported from knockin mouse and cell culture investigations in which the receptor was genetically modified to alter kinase-mediated signaling pathways. Specifically, they propose that the truncation creates an imbalance between AC/PKA versus PLC/PKA activation. They acknowledge that other mechanisms could be involved.

Duchatelet S, Ostergaard E, Cortes D, Lemaninque A, Julier C. Recessive mutations in *PTHR1* cause contrasting skeletal dysplasias in Eiken and Blomstrand syndromes. *Hum Molec Genet.* 2005;14:1-5.

Editor's Comment: *This is an interesting paper, not so much because of any firm conclusions about the mechanism involved since the authors provide no biological data; rather, it is because there are 3 distinct clinical phenotypes associated with mutations of the same gene. This study underscores the fact that many proteins have multiple functions that reflect different domains of the protein and that mutations of genes encoding these proteins can have quite different consequences depending upon which of these domain functions they disturb.*

William A. Horton, MD

Developmental Expression Patterns of Human Thyroid Transcriptional Regulators

To further the understanding of thyroid dysgenesis, the most common cause of congenital hypothyroidism, Trueba and colleagues examined the developmental expression patterns of human thyroid transcriptional regulators by in situ hybridization and immunohistochemistry in tissues obtained from legally terminated pregnancies. They focused on 3 factors: *PAX8, TITF1* (also known as *Nkx2a, Ttf-1* or *T/ebp*) and *FOXE1* (*Ttf-2* or *Titf-2*). These 3 factors lead to thyroid dysgenesis in knock-out mouse models and have been found to cause congenital hypothyroidism when mutated in humans. The *PAX8* gene was strongly expressed in median thyroid anlagen (from pharyngeal primordium) and laterally ectodermic region of the fourth pharyngeal arch, thyroglossal duct cells, ultimobranchial body and in later fetal follicular cells. It maintained follicular cell phenotype by activating thyroperoxidase, sodium/iodide symporter and thyroglobulin genes. The expression in thyroglossal duct cells suggested that the track was created by the migrating thyroid anlagen (rather than a pre-established pathway through which the thyroid migrated). This may explain why cells of thyroglossal duct remnants can differentiate into follicular cells to create follicle- and colloid-containing cysts. Additionally, *PAX8* gene had an extra-thyroid expression on the otic vesicle, central nervous system (midbrain-hindbrain boundary, spinal cord) and the developing kidney (metanephric blastema, ureteric bud and their derivatives). The clinical correlates of the *PAX8* gene have been found in congenital hypothyroid patients who also presented either unilateral renal agenesis or left-sided ureteropelvic obstruction. No *PAX8*$^{-/-}$ humans have been reported with bilateral renal agenesis (? lethal phenotype form). However, no CNS defects were seen in knock-out mice and heterozygote *PAX8* $^{+/-}$ humans have been detected.

The *TITF1* gene was weakly expressed in the median thyroid primordium and later fetal thyroid. The extra-thyroid expression was limited to the forebrain (hypothalamic floor and infundibulum, developing basal ganglia territory) and lung epithelial cells which became progressively restricted to distal branches, reducing surfactant production. The clinical correlates of the *TITF1* mutations were hypotonia and dyskinesia, changes in basal ganglia and pituitary gland and postnatal respiratory distress syndrome.

The *FOXE1* gene had a weak expression in thyroid primordium and gland throughout development and the extra-thyroid expression was seen in the thymus and in the oropharyngeal, tracheal and esophageal epithelium. The clinical correlates were seen in patients with thyroid dysgenesis and cleft palate as well as knock-out mice. There were no thymic or immunologic abnormalities yet reported. The thyroglobulin protein promoter contained binding sites for *PAX8, TITF1* and *FOXE1,* but thyroglobulin was not produced until the thyroid gland reached its final position.

Trueba SS, Auge J, Mattei G, et al. PAX8, TITF1, and FOXE1 gene expression patterns during human development: new insights into human thyroid development and thyroid dysgenesis-associated malformations. *J Clin Endocrinol Metab.* 2005;90:455-462.

Editor's Comment: *This paper is an excellent example of bench-to-bedside applications. It also points out that, despite the power of knock-out mouse models for explaining physiology, caution should be taken in extrapolating to humans as species differences occur. For a recent review of congenital hypothyroidism and its etiologies, see reference 1. Another genetic cause of congenital hypothyroidism, not listed in this paper or the review, is inactivating mutation of the gene encoding the TSH receptor; 2 siblings with compound heterozygosis had severe congenital hypothyroidism with apparent athyreosis, while their non-consanguineous, hemizygous parents had either normal thyroid function or compensated hypothyroidism with mild thyroid hypoplasia.*[2]

Adda Grimberg, MD

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Sex Differences in Patients Referred for Evaluation of Poor Growth

This study examines the sex difference in the rate of referral to a pediatric endocrinology center for evaluation of short stature or poor growth. The source of data was medical charts from all patients initially evaluated during 2001. After exclusion of those with a prior evaluation by a pediatric endocrinologist for treatment with growth hormone (GH) (n= 4), referral for evaluation of pituitary function secondary to brain disease or abnormality (n=15), and girls with known Turner syndrome (n=6), the medical records of 278 patients were available for analysis.

The table indicates multiple statistically significant disparities in anthropometric characteristics of boys and girls at the time of the initial visit to the pediatric endocrinologist.

Referrals for growth evaluation by gender (selected findings)

Variables	Boys (n = 182)	Girls (n = 96)	*P*
Gender	65%	35%	<0.0001
Median height at referral *	–1.9	–2.4	<0.01
Median deficit from mid-parental target height *	–1.3	–1.9	<0.001
Median time to referral (months) **	24	35	0.30
Age at referral			
< 9 years	57%	43%	<0.05
≥ 9 years	71%	29%	
Median height deficit at referral *			
< 9 years	–2.1	–2.4	0.48
≥ 9 years	–1.9	–2.4	0.01
Deficit from mid-parental height *			
< 9 years	1.5	1.8	0.34
≥ 9 years	1.2	2.1	<0.001
Organic disease+	15%	41%	<0.0001
Normal height referrals	38%	20%	<0.01
Familial short stature, constitutional growth delay, or both	72%	48%	

* z scores

** Calculated by subtracting age at first deviation across major percentiles from the age at first visit to pediatric endocrine center (growth curves were available for only 115 of 278 patients)

\+ Difference remains statistically significant after excluding girls with Turner syndrome (n = 9)

Grimberg A, Kutikov JK, Cucchiara AJ. Sex differences in patients referred for evalution of poor growth. *J Pediatr.* 2005;146:212-216.

Editor's Comment: *This is not the first epidemiologically-oriented study that has detected a sex difference in referral patterns.*[1] *Similarly, a survey demonstrated that pediatric endocrinologists were more likely to recommend GH therapy for boys with idiopathic short stature than for a girls with identical auxologic characteristics.*[2] *Converging evidence from these and additional studies replicate the societal bias that taller stature is more important in boys/men than in girls/women.*[3] *The fact that this bias is reflected in pediatric care is worrisome however. The under-representation of girls receiving growth evaluations raises the possibility of missed or late diagnoses. Alternatively, the over-representation of short boys in pediatric endocrinology referrals raises the possibility that health care has become complicit in societal prejudices along with the added burden to the patient of potential medical and psychological risks (recognized and unknown) as well as economic costs.*

This study raises an additional cause for concern: the majority of patients (59%) referred to one pediatric endocrinology clinic for a growth evaluation, arrived without plotted growth measurements. Other studies have shown that inaccurate height measurement tools are often used in primary care.[4] *What is needed is a return to fundamental practice, recommended by the American Academy of Pediatrics*[5] *of routine growth monitoring in primary care to differentiate healthy from pathological growth. Evidence of a strong sex bias in referral to a specialist suggests that clinicians (and parents) are possibly over-valuing "height" and possibly devaluing "growth" to the detriment of girls, in particular, and society at large.*

David E. Sandberg, PhD

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Visfatin – A New Visceral Fat Adipokine

Employing the method of differential display of expressed genes (by analysis of 8800 gene products utilizing cDNA probes) in paired samples of subcutaneous and visceral fat donated from 2 female volunteers, the investigators identified an adipokine that is synthesized primarily by visceral fat and termed this molecule "visfatin." They subsequently found that the visfatin had been previously identified as "pre-B cell colony-enhancing factor" (PBEF). This is secreted by the liver, bone marrow, and muscle, is a growth factor for early stage B lymphocytes, and down-regulates apoptosis of neutrophils. The investigators demonstrated that: 1) expression of PBEF/visfatin increased during adipocyte differentiation *in vitro* with increased secretion of this protein into medium; 2) plasma concentrations of visfatin correlated with the volume of visceral fat in humans and mice but not the quantity of subcutaneous fat; 3) plasma levels of visfatin increased as the amount of fat accumulated in a mouse model of obesity; and 4) visfatin values rose rapidly in mice ingesting a high-fat diet. Subsequently, the authors observed that intravenous administration of visfatin led to a dose-dependent decline in glucose concentrations without affecting insulin values in intact and diabetic mice. Complete knock-out of the visfatin gene was lethal. In heterozygotic (visfatin$^{+/-}$) animals, basal plasma glucose values were elevated, glucose tolerance was impaired, while there was no difference in size or insulin levels. *In vitro*, visfatin had several insulin-like actions including: enhancement of glucose uptake, suppression of glucose release, accumulation of triglycerides, and induction of gene markers of adipocyte differentiation (PPARγ, fatty acid synthase, adiponectin, and so forth). The investigators also showed that visfatin bound to the insulin receptor and induced its autophosphorylation, as well as phosphorylation of a number of downstream products consistent with induction of the insulin/insulin receptor signal transduction pathway. Most interestingly, they demonstrated that visfatin did not bind to the same segment of the insulin receptor as insulin (extracellular α subunit), although the binding site on the insulin receptor to which visfatin adheres was not identified. The authors concluded that visfatin has insulin-like effects and may be of physiological significance in the regulation of glucose homeostasis.

Fukuhara A, Matsuda M, Nishizawa M, et al. Visfatin: A protein secreted by visceral fat that mimics the effects of insulin. *Science*. 2005;307:426-430.

Editor's Comment: *This exciting discovery adds yet another factor to the many that regulate glucose and lipid homeostasis and to the list of adipocyte products that includes leptin, adiponectin, resistin, tumor-necrosis factor-α, and interleukin-6.[1] Although visfatin has many insulin-like qualities, its serum concentrations are lower than those of insulin and do not change acutely after eating. Inasmuch as visfatin is primarily secreted as the quantity of visceral fat increases, it may serve as a (less than optimal) compensatory mechanism for the deleterious effects of increased visceral adiposity. It is of interest that visfatin, like other non-peptidal small molecules, such as modified benzoquinones, can cross the plasma membrane and interact with and activate the insulin receptor tyrosine kinase.[2] These agents are active orally in animal models of type 2 diabetes mellitus; they increase insulin sensitivity and also exert other central and peripheral effects.*

Allen W. Root, MD

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GROWTH AND GROWTH HORMONE IN CHILDREN WITH NEUROFIBROMATOSIS TYPE 1

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INTRODUCTION

Neurofibromatosis type 1 (NF1), also known as von Recklinghausen disease, is an autosomal dominant, commonly inherited disease that affects one of every 3000 individuals.[1] The gene responsible for this condition has been isolated by positional cloning to chromosomal region 17q11.2. It spans over 350 kb of genomic DNA and encodes neurofibromin, a protein product of 2818 amino acids that is expressed in various tissues.[2] According to the National Institutes of Health Consensus Development Conference (Bethesda, Maryland, July 13-15, 1987), there are 7 key components of the disease (Table 1), at least 2 of which must be present in order to establish the diagnosis.[3]

Highlights In This Issue

E-Abstracts (Abstracts On-line)

From The Editor's Desk

In this issue the international contributing editors of *GGH* began abstracting papers for the readership. They reviewed the literature, selected important publications, abstracted them, and made insightful and important comments. Thus, with these contributions we continue to expand the reach of the journal. On behalf of everyone I thank them for their contributions and welcome them.

The lead article covers an important area that clinicians often encounter, namely evaluating the growth of patients with neurofibromatosis type 1. Growth alterations affect 13% to 24% of children with this disease, with more than 40% of adult NF1 patients reaching a decreased final height. Drs. Karantza and Geffner have written a notable review outlining the most pertinent issues of growth and growth hormone in NF1 patients. I am sure that this lead article will be tremendously useful and will constitute an important reference.

Additionally, the highlights of the 87th annual meeting of the Endocrine Society are summarized and 8 abstracts are presented in this printed *GGH*. There are 7 more abstracts posted on the web at www.GGHjournal.com. I trust you will enjoy and treasure this issue.

The next issue will include a historical review of growth hormone that commemorates the 20th anniversary of the FDA approval of recombinant human growth hormone and the launching of *GGH*—both made possible by Genentech.

Fima Lifshitz, MD

BACKGROUND

Endocrine disorders have been reported in approximately 1% to 3% of all NF1 patients. Pheochromocytoma is the most common endocrinopathy in adults with NF1, occurring in approximately 1% of patients.[4] In children with NF1, the most prevalent hormonal disorder is central precocious puberty (CPP), with a frequency of 3% compared to 0.06% in the

Growth, Genetics & Hormones is supported by an unrestricted educational grant from **Genentech, Inc.**

general pediatric population.[4-6] Delayed puberty has also been described, but its exact incidence has not been reported to date. Short stature (defined as a height that is equal to or more than 2 standard deviations [≥2 SD] below the population mean) has long been known to be a feature of NF1, affecting approximately 13% to 24% of prepubertal patients and >40% of adults.[7,8] Although short stature is the most common growth disturbance seen in patients with NF1, tall stature has also infrequently been described as a result of growth hormone (GH) hypersecretion linked to brain tumors.[9-15] It is the primary aim of this paper to summarize current knowledge on growth disturbances and GH secretion in children with NF1.

Table 1. Diagnostic Criteria

1. Six or more café-au-lait macules, the greatest diameter of which is >5 mm in prepubertal patients, and >15 mm in post-pubertal patients
2. Freckling in the axillary or inguinal region
3. Two or more neurofibromas of any type or one plexiform neurofibroma
4. Two or more Lisch nodules in the iris
5. Optic glioma
6. A distinctive osseous lesion such as sphenoid dysplasia or pseudoarthritis
7. A first-degree relative with NF1 diagnosed according to the preceding criteria

GENETICS OF GROWTH

The known molecular functions of the *NF1* gene and its protein, neurofibromin, could account for the short stature phenotype of NF1–affected individuals (Figure 1). Neurofibromin is a major regulator of the Ras pathway, a key signal transduction pathway which transmits mitogenic signals to the nucleus, and is expressed in many different tissues, including the brain. It contains a central domain related to Ras-specific guanosine triphosphatase-activating proteins (Ras-GAPs). It stimulates the intrinsic activity of Ras-GTPase and is involved in control of cellular growth and differentiation through down-regulation of Ras activity.[16] Mutations in the GAP-related domain of the *NF1* gene lead to increased levels of activated Ras and, thus, to increased downstream mitogenic signaling.[17] The NF1-conserved *Drosophila* homologue acts as a negative Ras regulator. Homozygous NF1 *Drosophila* mutants with 2 different mutations that result in lack of expression of NF1 protein are 20% to 25% smaller than flies of the parental strain, but are otherwise patterned normally. Their growth defect is rescued by expression of an *hsNF1* transgene, as well as by increasing cAMP-activated protein kinase A (PKA) expression, implying that both Ras and PKA interact in a pathway that controls overall growth.[18] Activated PKA has also been shown to play a critical role in stimulating proliferation of some cell types[19] and may physiologically contribute to body growth. Based on the *Drosophila* model, it could be postulated that alterations

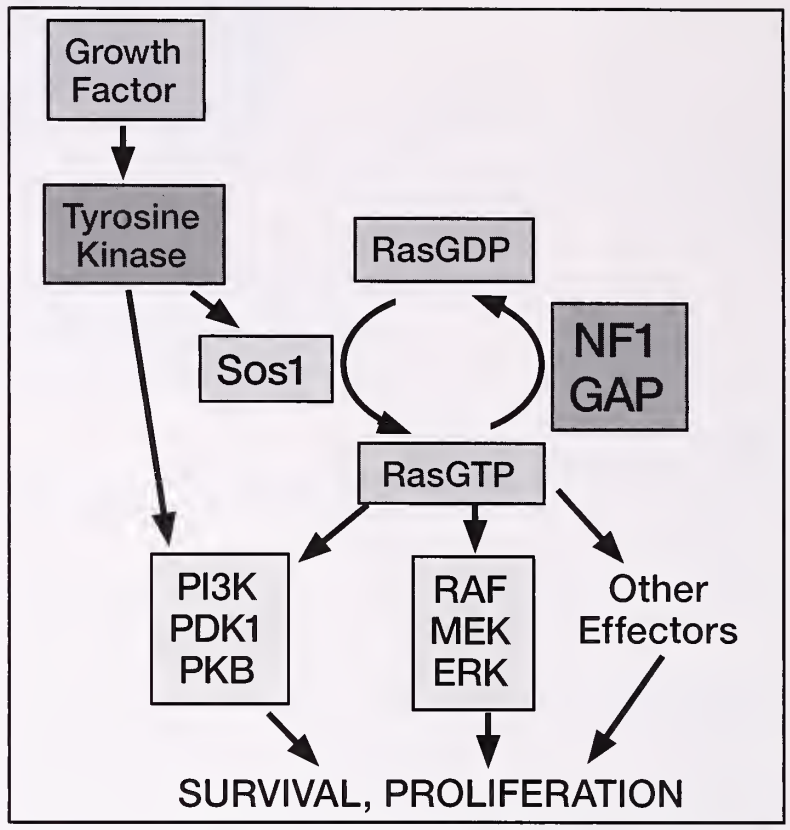

Figure 1. Cross talks among signaling pathways linked by GAPs. Neurofibromin (NF1) and p120RasGAP (GAP) control the hydrolysis of RasGTP. RasGTP is activated by growth factors via the exchange factor Sos1 and activates a number of effectors such as RAFkinase and PI3K, which in turn activate and phosphorylate the ERK and PKB kinases. In this way, GAPs can serve as key integrators of distinct signaling pathways. Reprinted with permission Donovan S, Shannon KM, Bollag G. *Biochim Biophys Acta*. 2002;1602:23–45. Copyright © 2002. Elsevier. All rights reserved.

in these pathways could result in smaller phenotypes in humans with NF1 as well.

GROWTH PATTERNS IN CHILDREN WITH NF1

Short Stature

Short stature associated with NF1 usually affects the skeleton symmetrically.[20] The etiology of short stature in patients with NF1 does not correlate with disease severity and is multifactorial, stemming from the disease itself or its complications. These complications may include problems that interfere with normal skeletal development, such as scoliosis[21,22] or deep plexiform neurofibromas, or the use of psychostimulant medications[23] for the treatment of attention deficit disorder,[24] which is a frequent behavioral problem in children with NF1. Risk factors for suboptimal growth are listed in Table 2.

Riccardi[20] suggested that short stature was an "all-or-none" phenomenon that affected only a subset of NF1 patients. Contrary to this suggestion, the National Neurofibromatosis Foundation International Database (NFDB) cross-sectionally analyzed the distribution of heights in 569 Caucasian North American children[25] with NF1 (Figure 2). Of note, the mean height SD score (SDS) among their patients was lower than that of the reference population. Thirteen percent of the NF1 patients fell >2 SD below the reference population mean, compared to only 2% of controls. They concluded that the distributions of stature

Table 2. Risk Factors Associated with Short Stature in Children with NF1

Suprasellar lesions
Surgery or radiotherapy for intracranial lesions
Growth hormone deficiency
Thyroid-stimulating hormone deficiency
Central precocious puberty
Delayed puberty
Scoliosis
Plexiform neurofibromas
Familial NF1
Familial short stature
Methylphenidate use for attention deficit disorder

are shifted and unimodal among NF1 patients. The NFDB provided NF1–specific growth charts (Figure 3). From a clinical standpoint, it is important to realize that deviations from the NF1–specific standards may indicate the additive effect of a specific disease feature, such as an optic glioma.

Clementi et al[26] also constructed NF1–specific growth charts in a study of 528 Italian patients with comparable stature centile curves to those of the NFDB. In this study, height velocity was normal during childhood for both sexes, whereas the pubertal growth spurt was slightly reduced in boys, but not in girls. During and post-adolescence, the 50th centile for NF1 patients overlapped with the 25th centile for normal subjects, but the 3rd centile was much lower in NF1 subjects than in normal subjects. There was no association of height impairment to disease severity. Carmi et al[7] prospectively evaluated parameters of growth, puberty, and final height in 89 children with NF1. Short stature was observed in 25.5% of patients during the prepubertal period, with a significant gradual reduction of relative height for age during puberty. Forty-three percent of patients had short adult height; of these, 58% had short stature attributable to familial NF1. Short adult height was more often attributed to central nervous system (CNS) pathology when the father was the affected parent, less when both parents were affected, and rarely when neither parent was affected. There was also a four-fold higher frequency of CPP among their patients compared to that observed in the general population, but the frequency of short stature remained the same even when patients with CPP were excluded. GH deficiency (GHD) as the cause of short stature was found only after neurosurgery and irradiation in a minority of short patients.

Tall Stature

Short stature is a cardinal feature in NF1; however, based on the stature distribution analysis of the NFDB, 24% of NF1 patients reside >2 SD above the reference population mean[25] (Figure 2). Carmi et al[7] reported tall stature in 4 of 89 patients with NF1, all without evidence of abnormalities in the GH axis. GH hypersecretion presenting as gigantism has rarely been described in children with NF1, and has always been associated with the presence of optic pathway gliomas (OPG).[9-15] In some of these patients, elevated prolactin was also observed.[12-14] Treatment of the OPG with surgery, radiation, and/or chemotherapy has resulted in a reduction in growth velocity and improved basal and stimulated GH levels in all cases. Bromocriptine[11] and, more recently, the somatostatin analogue, octreotide,[15] have also been successfully used in some tall NF1 patients. The mechanism of excess GH in these patients is not clear. There does not appear to be a direct secretory role of the tumor itself. Infiltration of the somatostatinergic pathways by the tumor leading to loss

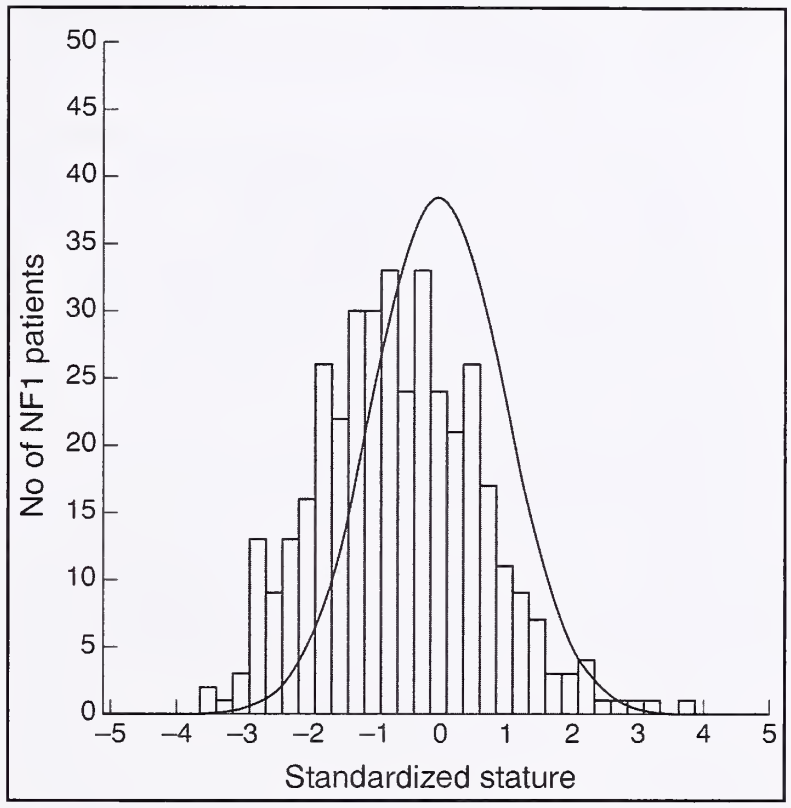

Figure 2. Distribution of sex and age standardized stature. NF1 patient measurements are from the National NF Foundation Database. Unaffected norms are from the National Center for Health Statistics and the Fels Institute. Reprinted with permission Szudek J, Birch P, Friedman JM. *J Med Genet*. 2000;37:933–938. Copyright ©2000. *British Medical Journal*. All rights reserved.

of somatostatinergic tone and, subsequently, increased GH release and loss of pulsatility, appears to be a possible mechanism in some cases.[9,10]

SUPRASELLAR LESIONS

Malignancy accounts for the development of significant morbidity and mortality in patients with NF1, including intracranial lesions, particularly suprasellar neoplasms. The first 6 years of life appear to be the period of highest risk for development of symptomatic tumors, the median age of detection being 4.2 years.[27] OPGs are the most frequent neoplasms, with an overall 19% incidence on routine magnetic resonance imaging (MRI) of the brain, but with a 7% *symptomatic* incidence.[28] Gliomas of the optic chiasm are reported to cause endocrinological disorders, especially CPP and GHD.

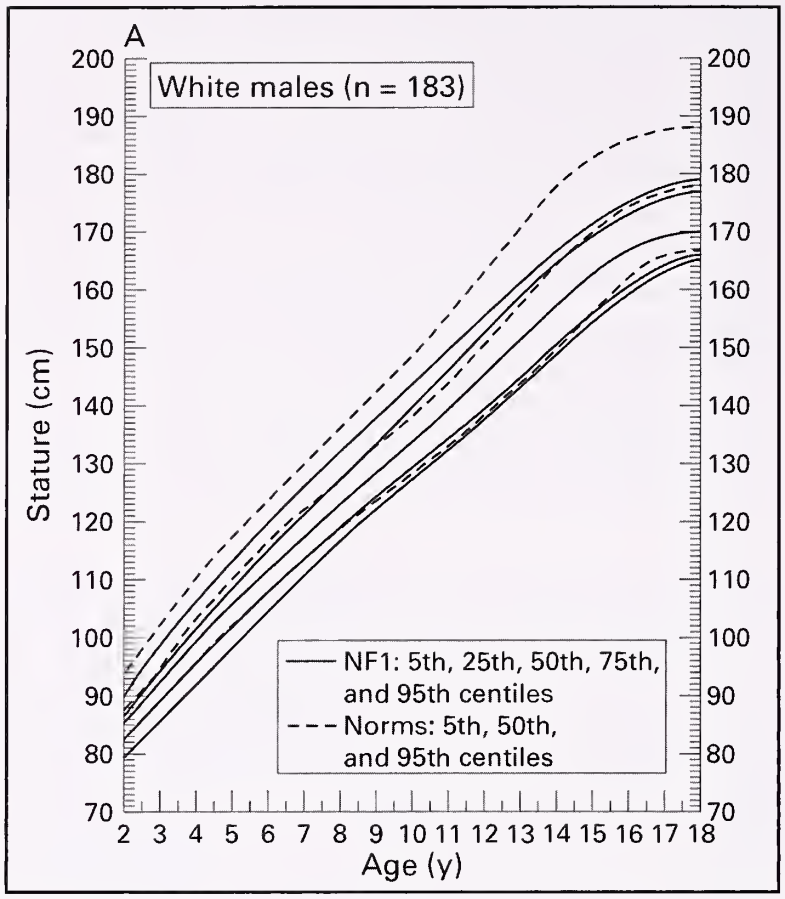

Figure 3. (A) Stature centiles in males 2-18 years. (B) Stature centiles in females 2-18 years. NF1 patient measurements are from the National NF Foundation Database and are denoted by solid lines. Unaffected norms are from the National Center for Health Statistics and are denoted by dashed lines. Reprinted with permission Szudek J, Birch P, Friedman JM. *J Med Genet*. 2000;37:933-938. Copyright ©2000. *British Medical Journal*. All rights reserved.

Central Precocious Puberty

In a study by Habiby et al[29] of 219 children diagnosed with NF1, 3% had CPP, all associated with OPG. This association also held true in all CPP patients in the study by Carmi et al.[7] However, in a study by Cnossen[30] of 122 children with NF1, the prevalence of CPP was the same as that previously reported; however, there was no evidence that OPG was a prerequisite for CPP, since only 1 of 3 children with CPP had an OPG at the time of diagnosis. Listernick et al[31] reported CPP in 5 of 17 children with an OPG and NF1, in contrast to no cases of CPP in a group of children with OPG and no features of NF1. Virdis et al[32] reviewed the records of 412 NF1 patients and also concluded that CPP is frequently—but not exclusively—associated with OPG. The above studies support an independent association of CPP and NF1 that cannot be solely attributed to OPG. A distinct feature of NF1-associated CPP is its slower rate of pubertal progression compared to CPP not associated with NF1. Whether treatment with gonadotropin-releasing hormone (GnRH) agonists is mandatory and/or efficacious in improving final height in the NF1 population remains under debate. However, there is general agreement that treatment should be offered in children manifesting signs of CPP at a young age and/or in those with a progressive decline in predicted final height.[33]

Growth Hormone Deficiency

GHD is an important complication in children with NF1, with the etiology in some patients remaining unclear. In the majority of children with NF1, GHD occurs primarily in those with an intracranial tumor who undergo intracranial surgery and cranial irradiation therapy. Indeed, in the study by Carmi et al,[7] using clonidine or insulin-induced hypoglycemia as GH secretagogues, all children diagnosed with GHD had a history of cranial surgery or irradiation. In a study by Pierce et al[34] of 24 patients with OPG, half of whom had NF1, GHD was found in 15 of 18 patients who were evaluated following treatment with radiotherapy. Huguenin et al[35] evaluated the relationship of adult height after cranial radiation for OPG to NF1, CPP, and GHD caused by the tumor itself or its management. Cranial irradiation resulted in GHD in 100% of cases. Reduced adult height resulted when there was GHD and CPP in the presence of NF1. In a retrospective review of the Pfizer International Growth (KIGS) database,[36] which is a database monitoring recombinant human GH (rhGH)-treated children, a total of 102 children with NF1 were identified, 43 of whom had an intracranial tumor. Ninety-two percent and 80% of the GH-tested patients with a cranial tumor had peak GH responses below 10 and 5 µg/L, respectively. Eighty-one percent and 56% responded below 10 and 5 µg/L, respectively, in the non-tumor group. The median GH peak response to stimuli (most commonly insulin-induced hypoglycemia or arginine) was significantly lower in the tumor group compared to the non-tumor group (3.0 vs 4.6 µg/L; $p<0.001$). However, Cnossen et al[30] reported a 2.5% prevalence of GHD in children with NF1 without an intracranial mass and *before*

surgical or radiation therapy for OPG, a frequency that is significantly higher than the 0.03% observed in the general pediatric population. An OPG was detected in 1 of 3 children with GHD, suggesting that GHD appears independently of the presence of OPG. In a study by Vassilopoulou-Sellin et al, [37] the incidence of GHD was investigated in 19 poorly growing children with NF1 and without other identifiable risk factors for shortness. Seventy-nine percent were diagnosed as having GHD on the basis of a peak GH response <10 µg/L after clonidine stimulation, and 42% had a peak GH level <5 µg/L, indicating a high frequency of profound GHD in this cohort. The causal mechanism of increased frequency of GHD in patients with NF1 remains to be elucidated. It is still plausible that despite the high-resolution capability of current MRI neuroimaging, cerebral abnormalities responsible for GHD are present, but not readily identifiable. Another possible explanation could be that there are abnormalities occurring at the cellular level, implicating the known molecular function of neurofibromin in signal transduction.[17]

Other Anterior Pituitary Hormone Deficiencies

Deficiencies of other anterior pituitary hormones such as thyroid-stimulating hormone (TSH) and adrenocorticotrophin (ACTH) have also been described in subjects with NF1 as a result of surgery and/or irradiation for intracranial tumors. Unrecognized hypothyroidism can account for poor growth, and unrecognized adrenal insufficiency can have potentially fatal consequences. Carmi[7] described 3 out of 6 children with NF1 and OPG who required thyroid hormone replacement after surgery and/or cranial irradiation. In the review by Huguenin et al,[35] no subject had TSH or ACTH deficiency prior to irradiation. However, 80% were found to be TSH-deficient and 17% were found to be ACTH-deficient after irradiation. Gonadotropin deficiency was variable with delayed or even arrested pubertal development in 43% of the patients, and low gonadotropin responses to GnRH were found in 60% of the patients evaluated.

GROWTH HORMONE REPLACEMENT

Efficacy

In a retrospective review of patients with NF1 from the KIGS database,[36] the outcome of 102 children treated with rhGH, at a mean dose of 0.18 mg/kg/wk with a mean duration of treatment of 2.7 years, was assessed. These included pre- and post-pubertal patients with and without intracranial tumors. The pretreatment median height SDS was –2.4 and the median height velocity was 4.2 cm/year. The median height velocity increased to 7.1 cm/year during the first year of treatment and remained above the baseline value during the next 2 years. The median height SDS increased from –2.4 to –1.9 in the first year and remained stable thereafter. There was no significant difference in the response to treatment between the tumor and the non-tumor groups, nor between those who had received radiation and those who had not. It is notable that the response to treatment was modest and less than that observed in patients with idiopathic GHD. However, the dose of rhGH given to patients with GHD was lower than that in other studies where an average dose of 0.30 mg/kg/wk was used, and it is likely that the growth velocity would have further declined if the patients had been left untreated. Vassilopoulou-Sellin et al[37] reported their experience with rhGH replacement therapy in a cohort, including children with NF1 and GHD without suprasellar lesions. This group of patients increased their annual growth rate (from a pre-treatment average) to 5 cm/year to 9 cm/year the first year, 8.3 cm/year the second year, and 6 cm/year during years 3 to 5 of rhGH therapy.

Safety

While therapy with rhGH has been shown to be safe, theoretical concerns remain that rhGH treatment may potentially increase an individual's risk of developing cancer *de novo* or increase the risk of recurrence of primary tumors and/or the incidence of second tumors in cancer survivors. Analysis of the KIGS database revealed recurrence of a primary CNS tumor and/or appearance of a second tumor in 5 of 102 rhGH-treated subjects[38] with NF1. Unfortunately, MRI neuroimaging was not performed in all patients prior to the start of rhGH treatment and, hence, definitive conclusions on the timing of malignancy presentation and its relation to rhGH therapy cannot be drawn. The natural history of OPG in children with NF1, as reported in previous studies,[39] suggests an incidence of tumor recurrence of 11% to 14%. There are also reports of a 30% recurrence rate of OPG after 10 years in NF1 patients under the age of 20 treated with surgery.[40] The occurrence of second intracranial tumors has also been frequently reported in children with NF1 and OPG. Hochstrasser[41] and Kuenzle[42] reported second tumors in 21% and 52%, respectively, during 9 years of follow-up. Based on the results of the above studies and clinical observations, there does not appear to be an increased risk of primary tumor recurrence nor development of a second malignancy in children with NF1 treated with rhGH.[43] However reassuring the data may be, continuous surveillance for all NF1 individuals treated with rhGH is mandatory.[44]

Progression of NF1 Features

It is well documented that café-au-lait macule size increases during puberty.[45] It is also known that neurofibromas increase both in size and in number in pubertal patients. Superficial growth of neurofibromas can lead to underlying segmental hypertrophy, whereas deeper structure invasion of the spine and paraspinal areas can create anatomical problems, the most dangerous of which is spinal cord compression. Whether rhGH treatment can accelerate or augment the growth of these lesions with harmful sequelae remains of concern. Indeed, 13% of the NF1 patients in the KIGS database,[36] many of whom

were pubertal, had changes in café-au-lait macules and neurofibromas. There are no reports that the increase in disease progression was accelerated secondary to rhGH, although one patient had an increase in the size of a prelumbar mass thought to be a neurofibroma. Cnossen et al[30] reported no growth of neurofibromas that could be ascribed to rhGH replacement in their patient population. The above results are reassuring; however, until larger-scale observations become available, close monitoring of the growth of neurocutaneous lesions is still warranted in rhGH-treated NF1 patients.

CONCLUSION AND SPECULATION

Short stature is a well-recognized manifestation of patients with NF1, although its etiology is not fully understood. Insight as to what represents a normal pattern of growth for individuals with NF1 has been gained through the generation of NF1-specific growth charts using information from the NFDB. It is apparent that most children with NF1 grow normally until puberty. Thereafter, their height velocity is diminished compared to their healthy peers, leading to a final height significantly below their predicted genetic target. Disease-specific features, such as scoliosis and extensive neurofibromas, can further compromise final adult height. Suboptimal growth (using the NF1-specific growth charts) is also a compelling argument to look for disease-related complications such as malignancies, the most common being OPG. These tumors are frequently the cause of CPP which if present, may further compromise final height. It is also important to be aware that the increased incidence of CPP in patients with NF1 cannot solely be attributed to the presence of OPG, as CPP may occur in this setting without any tumor. Treatment of symptomatic OPG with radiation, surgery, or chemotherapy may result in decreased final height by causing damage to the hypothalamic-pituitary region and connections thereof, resulting in one or multiple pituitary hormone deficiencies, most often GH. Recent evidence of an increased incidence of isolated GHD without identifiable risk factors in children with NF1 suggests that screening is mandatory when no plausible alternative explanation accounts for a suboptimal growth velocity. Children with NF1 have a modest, although significant response to GH treatment. Current knowledge suggests that such treatment does not influence the progression of any of the features of NF1, including the incidence of recurrence of primary or the development of secondary intracranial tumors. Hence, it appears that the use of GH is efficacious and safe in children with NF1 and GHD, although continuous vigilance is necessary. The discovery of neurofibromin, with its multiple actions on signal transduction and control of cellular growth, has shed light onto aspects of the molecular biology of the disease. Further analysis and exploration of the *NF1* gene action and the effects of its mutations may help to elucidate the cellular pathways leading to the phenotypic features (including growth disorders) of neurofibromatosis.

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ABSTRACTS FROM THE LITERATURE

Summary Highlights: Endocrine Society 87th Annual Meeting in San Diego, June 4-7, 2005

The full ENDO 2005 program may be viewed online at www.endo-society.org. Summarized here are some highlights of interest to the editor.

Growth

A new cause of short stature was reported by Olney et al from Jacksonville, Cleveland, and Christchurch (OR43-4). They described **acromesomelic dysplasia**, Maroteaux type (AMDM), a form of dwarfism characterized by marked short stature with short arms and legs. These patients had a mutation in the natriuretic C-type peptide (CNP) receptor B gene that prevents cells from exerting the action of this peptide to enhance growth, despite very elevated CNP levels. Patients with AMDM were homozygous for the gene mutation, whereas family members with one single gene copy mutation did not exhibit the syndrome but had short stature and were about 9.5 cm shorter and had an elevated CNP level 4 times higher than other family members who did not have the CNP mutation. Researchers estimated that approximately 1 in 700 people have a mutation in this gene. Thus, this alteration may be present in up to 1.3% of short stature children.

Naturally conceived children have different growth patterns and lipid profiles than those of children conceived by **in vitro fertilization** (IVF), as reported by Miles et al from Auckland (OR54-6). They studied 110 children, ages 4 to 10 years, 50 of them were born following IVF. The IVF children were taller and had higher growth-promoting hormones IGF-I, IGF-II and IGFBP3 and had a more favorable cholesterol profile than those conceived naturally.

Countering previous data, Maghnie et al from Pavia, Milano, Parma, Rome, and Cagliari (P1-504) studied patients with **multiple pituitary hormone deficiencies** (MPHD) and those with **isolated growth hormone deficiency** (IGHD). They reported that both types reached the same adult height. They studied 49 patients with MPHD and 39 with IGHD who were diagnosed at a median age of 7 years and treated with GH. The median adult height did not differ among the 2 groups. Adult height of MPHD patients was positively correlated with both the time period of GH treatment and with height at the time of diagnosis. The adult height of IGHD patients was positively correlated with height at diagnosis and with pubertal height gain.

A simple, compact **inhaler** showed promise in easing **delivery of human growth hormone** (hGH) as safely and effectively as by injection. Chipman et al from Indianapolis, Cambridge, and London (OR33-3) treated 12 healthy males, 21 years to 36 years of age, in a cross-over design, with hGH given subcutaneously or by inhaler. Blood levels of GH achieved with either method of administration were similar among both groups. Mild side effects were similar and infrequent in the 2 groups. This study showed evidence that inhalation of large proteins may produce blood concentration-time profiles and variability levels similar to those obtained by hGH injection.

Geffner et al from Los Angeles, Stockholm, Prague, London, and Antwerp (P2-505) reported patients with **childhood-onset growth hormone deficiency** (CO-GHD), who discontinued treatment after reaching adult height, but later needed additional GH therapy. They stated that GHD patients should resume treatment as soon as possible. Data from 210 patients with severe CO-GHD followed in the Pfizer International Growth and Metabolic database (KIGS and KIMS), who were off GH for more than 6 months during transition from childhood to adulthood therapy were studied. There was a significant difference in IGF-I, cholesterol, and triglyceride levels and quality-of-life scores after GH was discontinued. The poorest results were among those with longer intervals without GH. Thus, after retesting to confirm persistence of GHD, GH treatment should be resumed promptly in adults with a history of CO-GHD.

Obesity

Westphal et al from Ulm, Leipzig, Luebeck (P2-149) reported a novel link describing the connecting signaling pathways in adipose tissue with arterial blood pressure. The connection was the **JAK/STAT pathway** that inhibited 3-adrenergic crosstalk which down-regulated expression of angiotensin II, thus contributing to the regulation of the renin-angiotensin system. This crosstalk may represent the molecular link between **obesity and hypertension**.

Flint et al from Philadelphia (P1-705) determined that overweight children need repeat evaluations of their **glucose tolerance**, as their ability to metabolize glucose changes over time. Overall, 6 of the 44 children studied demonstrated deterioration in glucose tolerance in 15 months. In contrast, there was no significant change in the body mass index, HOMA-1R, cholesterol, and triglyceride levels of these 6 children over time. Longitudinal evaluation by oral glucose tolerance testing was necessary to detect worsening glucose metabolism.

According to de Zegher et al from Barcelona, Cambridge, and Leuven (OR34-4), **small for gestational age** (SGA) infants who have rapid **catch-up weight gain**, present with excess body fat by 2 to 3 years of age, and

may therefore already be on the path to type 2 diabetes (T2DM) later in life. The authors compared a group of toddlers with body composition measurements by DEXA, who were born small (SGA) and who normalized their weight during infancy, with another group of toddlers of average size at birth (AGA) and with normal weight in infancy. The SGA infants accumulated more adipose tissue than lean body mass; fat was deposited principally in the abdominal region and noticed shortly after completion of their catch-up growth. The path from early growth restraint to insulin resistance and later T2DM emerges early in life.

Diabetes

For the first time a developmental gene was discovered capable of reversing **autoimmune diabetes mellitus** in mice by Yechoor and Chan et al from Houston and Otsu (P1-10). The researchers treated mice with type 1 autoimmune diabetes (NOD/LtJ) with a single intravenous infusion of an **islet cell developmental gene** (HDAd-ND and HDAd-BTC). This reversed the disease and normalized the blood sugar levels. The *in vivo* gene with nueroD along with betacellulin reversed the autoimmune process and restored insulin secretion. This is a promising and interesting alternative to islet cell transplantation.

According to Milanesi et al from Winston-Salem and Padova (P1-14), **amniotic fluid stem cells** showed promise in treating **type 1 diabetes** by functional regeneration of pancreatic islets. The researchers found that mouse amniotic fluid stem cells, taken from pregnant mice, could induce pancreatic differentiation and formation of islet-like clusters that expressed insulin in streptozotocine (STZ)-treated NOD/SCID mice. When given the stem cells, these mice showed the same number of islets as the healthy mice. Furthermore, the treated mice maintained normal blood glucose levels and their pancreas' showed normal islets that co-expressed insulin.

Steroids

This report by Yazawa et al from Fukui and Saitama (P1-330) showed that **stem cells from adult bone marrow** were able to create **cells in the testis and in adrenal glands**. Mesenchymal stem cells or marrow stromal cells were found to be engrafted and differentiated into steroidogenic cells that were indistinguishable from Leydig cells when transplanted into immature rat testes. Because adult stem cells can be easily obtained from adult bone marrow by simple aspiration, these findings may be of great potential as a stem cell resource that may be used clinically for diseases associated with steroid hormone-producing alterations.

Fima Lifshitz, MD

Caffey Disease is a Type I Collagenopathy

Caffey disease (OMIM 114000), also known as infantile cortical hyperostosis, is characterized by spontaneous episodes of subperiosteal new bone formation typically involving the diaphyses of long bones, mandible, and clavicles in young children. It is associated with acute inflammation of soft tissues and can lead to profound alterations in the shape and structure of affected bones. It often exhibits an autosomal dominant pattern of inheritance with substantial variation in severity.

A group headed by Jüppner undertook genome-wide linkage studies to map the Caffey disease gene locus in 3 unrelated families. Their search led them to chromosome 17q21 and eventually—to their surprise—to the *COL1A1* locus, which encodes the α 1 chain of type I collagen. Affected individuals in all 3 families had the identical mutation: an arginine to cysteine substitution at position 836 (R836C) placing it in the carboxy portion of the triple helical domain of the collagen molecule. About one-fifth of family members in whom the mutation was detected had no clinical features consistent with previous reports of reduced penetrance for the condition.

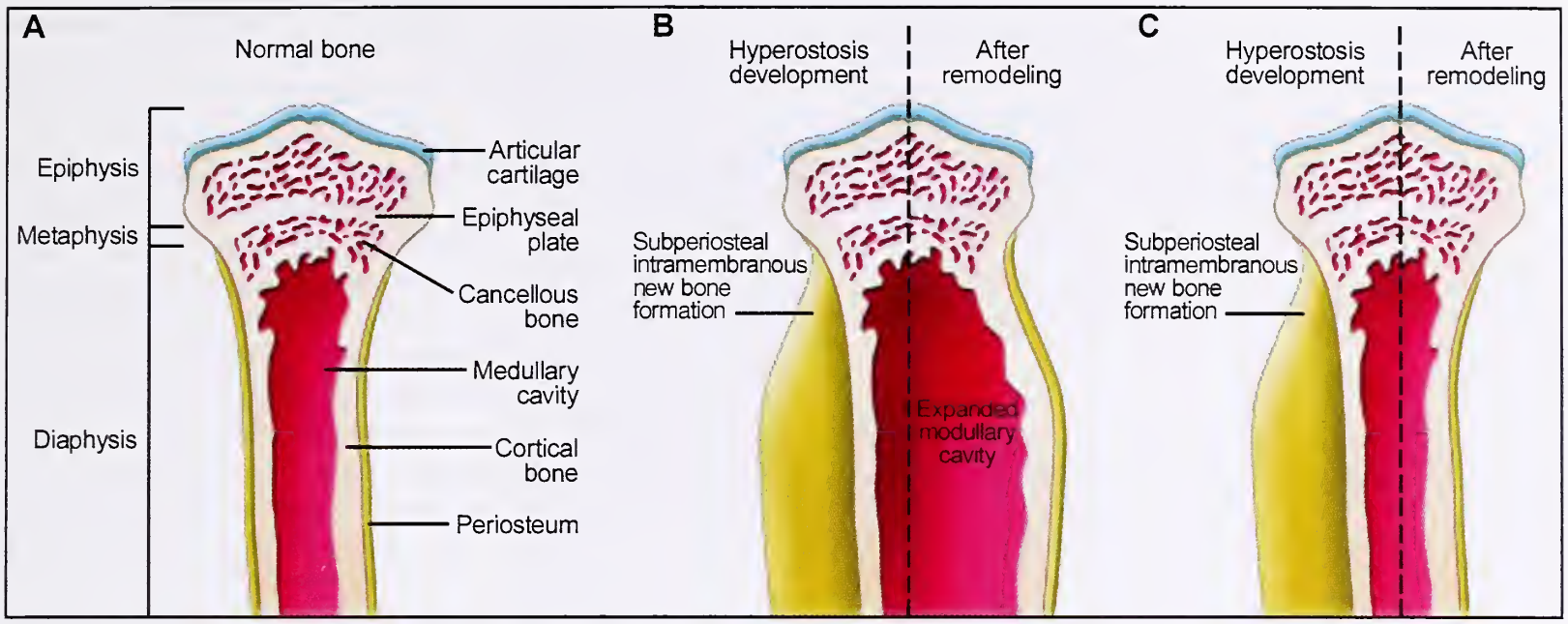
Mutations of the *COL1A1* are typically associated with osteogenesis imperfecta (OI) and, to a lesser extent, with Ehlers-Danlos syndrome (EDS). The affected members of these families did not display clinical signs of OI. They lacked gray-blue sclerae, dentinogenesis imperfecta, premature hearing loss, and short stature. Although bone fractures were relatively common in one family, they were considered within the range of normal. One affected member in this family had normal bone densitometry studies.

Several affected family members had joint hypermobility and abnormally soft and hyperextensible skin suggestive of mild EDS. Electron microscopy of a skin biopsy from one of these patients revealed that collagen fibrils varied more in size and shape and were less densely packed than normal. Collagen biosynthetic studies of fibroblasts from this patient showed abnormalities consistent with the presence of cysteine residues in the mutant type I collagen chains.

Perhaps most interesting, as addressed by both Gensure et al and in an accompanying comment by Glorieux,[1] is how one explains the episodic nature of this condition by a mutation in an extremely abundant structural protein present in bone and neighboring tissues (Figure). Although both raise several interesting possibilities, they also conceded that the question remains open and will require further investigation.

Gensure RC, Mäkitie O, Barclay C, et al. A novel COL1A1 mutation in infantile cortical hyperostosis (Caffey disease) expands the spectrum of collagen-related disorders. *J Clin Invest*. 2005;115:1250–1257.

Editor's Comment: *It was not surprising to learn that OI and some forms of EDS are allelic disorders given the overlap in some of their features. However, it is surprising*



Schematic illustrating normal and exuberant bone formation. (A) Representation of a growing bone. Growth in length is achieved by endochondral bone formation adding cancellous bone in the metaphyseal area. Gain in diameter comes from subperiosteal new bone apposition by intramembranous bone formation. The periosteum is an envelope of fibrous connective tissue that is wrapped around diaphyses. The size of the marrow cavity is controlled by a combination of bone apposition and resorption at the endocortical surface. (B and C) In ICH/Caffey disease, hyperostosis develops by exacerbated subperiosteal intramembranous bone formation triggered by local inflammation (left side of B and C). In the remodeling phase, the excess of bone tissue is resorbed either at the endocortical surface, leading to an expansion of the marrow cavity and a more persistent deformity (right side of B), or at the exocortical surface, with no effect on the size of the marrow cavity (right side of C). Reprinted with permission Glorieux F. *J Clin Invest*. 2005;115:1142–1144. Copyright ©2005. ASCI. All rights reserved.

to find Caffey disease in this group. Even though there appears to be some clinical overlap with mild EDS, the inflammatory and episodic nature of Caffey disease makes it quite distinct. As both Gensure and Glorieux[1] point out, some of the differences in clinical phenotype may be due to the fact that the mutation reported here does not involve a glycine residue as do most OI mutations. Collagen glycine mutations are thought to disrupt the formation and stability of the collagen triple helix, which is responsible for the structural properties of collagen. The Caffey disease mutation affects an arginine residue, which interestingly has been reported in 2 unrelated patients with classic EDS. As both also note, administration of prostaglandin E to infants with congenital heart disease sometimes causes local hyperostosis similar to episodes of Caffey disease, raising the possibility that this substance somehow mediates the pathologic events. If so, it remains to be determined how abnormal type I collagen sets the stage for inflammation and reactive bone formation.

William A. Horton, MD

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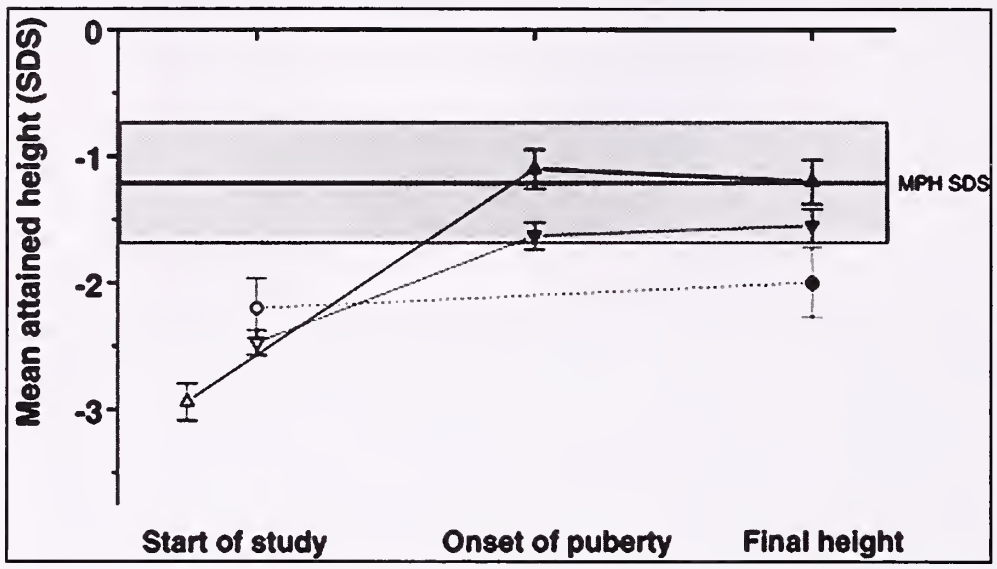
Final Height in SGA Children Treated with Growth Hormone

Dahlgren and Wikland report for the Swedish Study Group for growth hormone (GH) treatment of short children born small for gestational age (SGA). The final height (FH) achieved in 77 patients treated with exogenous GH (33 µg/kg/day starting prior to puberty and continuing until growth was less than 1 cm/year) was compared with data from GH treatment trials. Data were compared with data from a group of 34 short untreated SGA children. All children were born SGA (–2 SDS from mean for gestational age) for weight, height, or both, during the years 1973 to 1984. Only data from prepubertal children were analyzed. Two groups were identified: those who received GH more than 2 years before the onset of puberty (Group 1) and those who received GH beginning less than 2 years from the start of puberty (Group 2). A subset of 28 children were randomized to receive either 33 or 66 µg/kg/day during puberty. Children were excluded from the analysis if they had chromosomal abnormalities, serious malformations, chondrodysplasia, maternal history of alcohol or substance abuse, or a condition requiring chronic medical treatment. The projected FH was compared with height of the reference population in Sweden and the gain in FH as the projected adult height in SDS minus the achieved adult height in SDS. Maternal and paternal heights were compared with reference values and mid-parental height (MPH) in SDS. Arginine-insulin GH stimulation tests were performed in all but 2 children; 37% of patients failed to achieve maximal serum GH stimulation values of 5.3 µg/L (cut-off for severe growth hormone deficiency at the time of diagnosis).

The mean FH of the entire group was –1.2 SDS, reaching the mean MPH of –1.2 SDS, and 86% of the children achieved a FH within their target height (within 1 SDS from their MPH). In the untreated, comparison group, only 52% achieved a FH within their target height ($p<0.001$). Although the mean height gain for the entire

group was 1.3 SDS ± 0.8, those treated for more than 2 years prior to the onset of puberty had a gain of 1.7 SDS ± 0.7, while those treated less than 2 years prior to the onset of puberty had a smaller gain of 0.9 SDS ± 0.7. The growth responses were most pronounced among those treated the longest prior to puberty. No differences were seen in FH among the subset of children who received the higher doses of GH during puberty (Figure).



The prepubertal and pubertal height gain (SDS) in the two GH-treated groups, expressed as mean and SE: *regular triangles* = treated >2 y before puberty, and *inverted triangles* = treated <2 y before puberty. Attained height in the untreated group is shown as *circles* and *broken line*, expressed as mean and SE. Mean MPH ± 0.5 SD, is shown as *shadowed area*.

The authors discussed the importance of treating SGA children at as early an age as possible and the effects of continuing that therapy until growth is complete. They also noted that the therapy was well tolerated with no drug-related adverse events. They emphasize that differences between their study results and those of others may relate to the long duration of GH treatment in their cohort. They concede that a broad range of height gain was observed and that this suggests that individualized dosing may be appropriate. They conclude that younger, shorter, and lighter children at the start of GH treatment have better growth responses, are taller at the onset of puberty, and achieve a better FH.

Dahlgren J, Wikland K on behalf of the Swedish Study Group for Growth Hormone treatment. Final height in short children born small for gestational age treated with growth hormone. *Pediatr Res.* 2005:57:216–222.

Editor's Comment: *This is an interesting and potentially important study of the long-term effects of GH therapy on FH in children born SGA. Many pediatric endocrinologists are faced with the decision whether or not to recommend GH therapy for young short children born SGA. Parents often ask if there is any harm in delaying treatment until the child is older, perhaps at an age when the benefits of daily injections might be more understandable. This manuscript suggests that delaying GH treatment in such children is not in their best interest and that maximal benefits are associated with early prepubertal therapy.*

It would have been interesting to know whether or not the children who had a GH deficiency by stimulation testing (33%) grew better than those who made sufficient amounts of GH. Since 50% of the comparison group achieved FH at their MPH without any GH therapy, one wonders if the difference in outcomes between the comparison group and the treated group could be accounted for by the increased growth rates of treated GH-deficient SGA children.

Finally, it is important to note that although no adverse events related to GH therapy were reported, the authors did not report which potential side effects were screened for and what type of testing was performed to assure that they did not occur. Specifically, it would be very important to know how glucose intolerance and/or insulin resistance was monitored in these children. This editor would caution pediatric endocrinologists who opt for long-term GH therapy for short SGA children to monitor them carefully and repeatedly for potential side effects.

William L. Clarke, MD

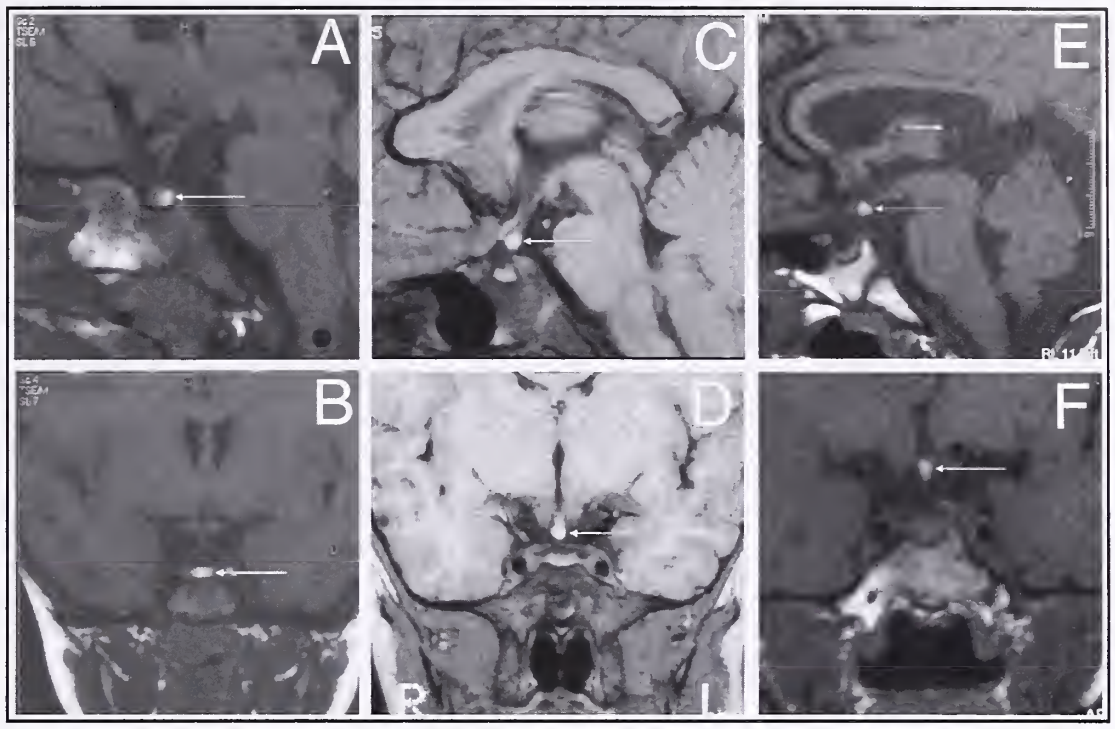
Is Growth Hormone Deficiency in Ectopic Neurohypophysis Permanent?

Anatomical abnormalities of the hypothalamic-pituitary axis, as detected by cerebral magnetic resonance imaging (MRI) in children with isolated growth hormone deficiency (GHD) or multiple pituitary hormone deficiencies (MPHD) are a landmark of a group of patients with hypopituitarism. Leger et al reported a prospective study of a group of such 18 patients who had in common MRI markers of ectopic neurohypophysis with defects of the pituitary stalk. The researchers followed the patients until adulthood, after completion of GH treatment. The initial diagnosis of GHD was based on a GH peak of <10 µg/L after provocative stimuli. At retesting, the same criteria were applied, but GHD was considered as severe if the peak value was <5 µg/L. The important finding at reevaluation was the presence of normal or only partially deficient GH secretion with a peak value of >5 µg/L in 7 patients; 6 out of whom had isolated GHD. Among the 11 patients with severe GHD at retesting, only one had isolated GHD. Therefore, MPHD, regardless of etiology, was a strong predictor of permanent GHD after adolescence.

The MRI structure of the hypothalamic-pituitary axis differed among both groups. It should therefore be

recalled that the main anatomical finding in the so-called pituitary stalk interruption syndrome is the ectopic location of the bright spot of the neurohypophysis. This spot may be located in its upper position at the median eminence or at a lower level along the pituitary stalk with a hypoplastic anterior pituitary. The ectopic neurohypophysis was found at the median eminence level in 10 out of 11 patients with permanent, severe GHD. In contrast, it was located along the stalk in all but one of the patients with normal or partially reduced GH response at retesting.

The authors concluded that increased GH secretion may be observed in adult patients with less severe MRI anatomical defects. These individuals need to be retested at the completion of GH treatment. In contrast, the patients who persisted with severe GHD formed a subgroup with their neurohypophysis at the median eminence with lack of or poor visibility of the pituitary stalk. Retesting may not be necessary in these patients, especially if there is MHPD.



Cerebral MRI (T1-weighted images). A, Sagittal slice; B, coronal slice; normal morphology of anterior pituitary and pituitary stalk is seen. The hyperintense signal of the posterior pituitary is in the normal location. C, Sagittal slice; D, Coronal slice; a normal anterior pituitary with a thin pituitary stalk is seen. The ectopic posterior pituitary hyperintense signal is located along the stalk (at a proximal level of the pituitary stalk; *arrow*). E, Sagittal slice; F, coronal slice; hypoplastic anterior pituitary with no visible pituitary stalk after gadolinium injection. The ectopic pituitary hyperintense signal is at the median eminence (*arrow*). Reprinted with permission Leger J, et al. *J Clin Endocrinal Metab*. 2005;90:650-656. Copyright ©2005. The Endocrine Society. All rights reserved.

Leger J, Danner S, Simon D, Garel C, Czernichow P. Do all patients with childhood-onset growth hormone deficiency (GHD) and ectopic neurohypophysis have persistent GHD in adulthood? *J Clin Endocrinol Metab*. 2005;90:650–656.

Editor's Comment: *The authors studied a group of non-acquired GHD patients identified by MRI-detectable anatomical defects of the hypothalamic-pituitary axis. This type of patient is of theoretical as well as practical interest. It was first hypothesized that all patients with such MRI-detectable defects would show permanent GHD and eventually be candidates for life-long GH therapy. It is now shown that a subgroup (approximately 40%) with isolated GHD in childhood may appear as normal, or moderately affected, at retesting after growth is completed. Furthermore, the patients with GHD appear to present an anatomical defect detectable in the MRI which can be considered as less severe: the pituitary stalk is eventually visible and the neurohypophysis has partly "migrated" downward. These findings may help predict a more favorable outcome. In contrast, those presenting with MPHD with an ectopic neurohypophysis located in the median eminence usually present persistent GHD into adulthood. However, a word of caution is necessary as some of the patients with isolated GHD may develop other pituitary defects at any age. They require a lifetime follow-up, even if GH secretion has apparently returned to normal. These data should be considered for future guidelines of GH treatment.*

The pathogenesis of the pituitary stalk interruption syndrome with pituitary insufficiency remains unknown in most cases. This defect has been reported in some patients with identified molecular defects of transcription factors controlling the early pituitary development.[1-3] *Although it was not the scope of this study, it seems important to not consider pituitary dysfunction as a stable condition. As also shown in other studies, the switch from isolated GHD to multiple defects remains possible at any age.*[4,5] *It will be of great interest to follow the patients who had an apparent recovery reported by Leger et al to document their reproductive function. Finally, patients with this illness are candidates for genetic studies and long-term follow-up to provide a more complete and eventually significant description of their hypothalamic-pituitary function throughout life.*

Raphaël Rappaport, MD

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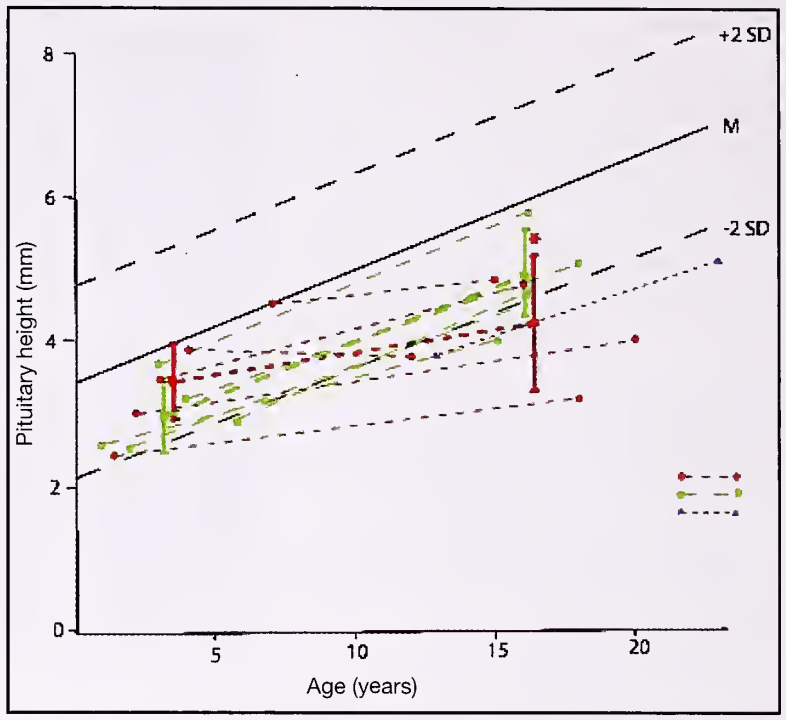
Familial Isolated Growth Hormone Deficiency Type II: Not So Isolated After All

Isolated growth hormone deficiency (IGHD) is thought to be familial in 5% to 30% of cases. Familial IGHD is categorized into 4 types: IA is autosomal recessive with absent endogenous GH; IB is autosomal recessive with decreased GH; type II is autosomal dominant with decreased GH; and type III is X-linked with decreased GH. Type II IGHD results from *GH-1* gene mutations[1] that lead to missplicing and subsequent loss of exon 3; the resultant 17.5-kDa GH variant acts as a dominant negative inhibitor of the normal 22-kDa GH isoform (from the wild-type allele) by disrupting the Golgi apparatus, impairing trafficking of GH and other hormones, and reducing stability of the 22-kDa GH isoform.

Mullis and colleagues studied 57 subjects from 19 families with type II IGHD resulting from different splice site and missense mutations in *GH-1*. Thirty-three had received GH treatment, and 24 were untreated. Those who had been treated during childhood stopped treatment for 2 months when reaching near adult height and underwent pituitary retesting; the untreated subjects underwent similar testing. Several interesting findings arose. First, subjects with a splice site mutation in the first 2 bp of the third intron (5´IVS +1/+2 bp) seemed to have a worse phenotype than those whose splice site mutation occurred in the 5th or 6th bps of the same intron (5´IVS +5/+6). The former had lower mean serum cortisol and ACTH concentrations and were more likely to have lower TSH levels. They also had a significantly smaller pituitary height (–2.59 SDS vs –1.56 SDS, $P<0.01$) when reaching adult height. One patient with a missense mutation (P89L GH) also presented with ACTH and TSH deficiencies, and another (R183H GH) had a small pituitary size at age 73 years.



Pituitary height in affected GH-treated subjects at diagnosis as well as at the end of growth. The age-dependent heights of the adenohypophysis, which was determined in a strict midline positioned sagittal scan, are shown. Because MRI was performed at different ages and the size of the normal pituitary increases with age the –2.0 and +2.0 SDS are shown as *lines*. In each subject 2 measurements were performed: at the beginning/diagnosis and at near AH after the GH treatment was stopped for 2 months. *Green/closed squares*, Patients with 5´IVS-3 +5/+6 bp splice site mutation; *red/closed circles/dots*, patients with 5´IVS-3 +1/+2 bp splice site mutation; *blue/closed triangle*, R183H GH. *, $P < 0.01$. Reprinted with permission Mullis PE, et al. *J Clin Endocrinol Metab.* 2005; 90:2089–2096. Copyright ©2005. The Endocrine Society. All rights reserved.

The authors concluded that the phenotype was partially genotype-related. On one hand, children with splice site mutations were younger at diagnosis (mean age 3 years) than those with missense mutations (mean age 9.3 years), and the splice site mutation in the first 2 bps of intron 3 presented with more pituitary dysfunction in adulthood than mutation in bps 5 or 6 of the same intron. However, there was still considerable phenotypic

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variability among individuals within the same family with the same mutation. Consistent with transgenic mouse models, it seems the phenotype is dose-dependent (ie, the ratio of mutant 17.5 kDa GH to wild-type 22 kDa GH). Transgenic mice with high-copy number IGHD II also developed pituitary hypoplasia and multiple hormone deficiencies (prolactin, TSH and in males only, LH).

Mullis PE, Robinson ICAF, Salemi S, et al. Isolated autosomal dominant growth hormone deficiency: an evolving pituitary deficit? A multicenter follow-up study. *J Clin Endocrinol Metab*. 2005;90:2089–2096.

Editor's Comment: *I agree with the authors that the most important lesson from this study is the need for long-term monitoring of pituitary function in patients with type II IGHD. Interestingly, the hormonal deficiencies and pituitary hypoplasia manifested later. The difference in pituitary size among patients with the 2 splice site mutations (+1/+2 vs +5/+6) was not significant at the time of diagnosis (–1.1 and –1.5 SDS, respectively), but became significant by the time near adult height was reached (–2.59 and –1.56 SDS) (Figure). Although type II IGHD is supposed to have isolated GHD by definition, the onset of additional pituitary deficiencies in adulthood warrants attention. This is reminiscent of the finding of central adrenal insufficiency in adults who had been treated for idiopathic GHD in childhood.*[2] *Unrecognized and under-treated adrenal insufficiency contributes to the increased mortality of individuals with GHD.*

Adda Grimberg, MD

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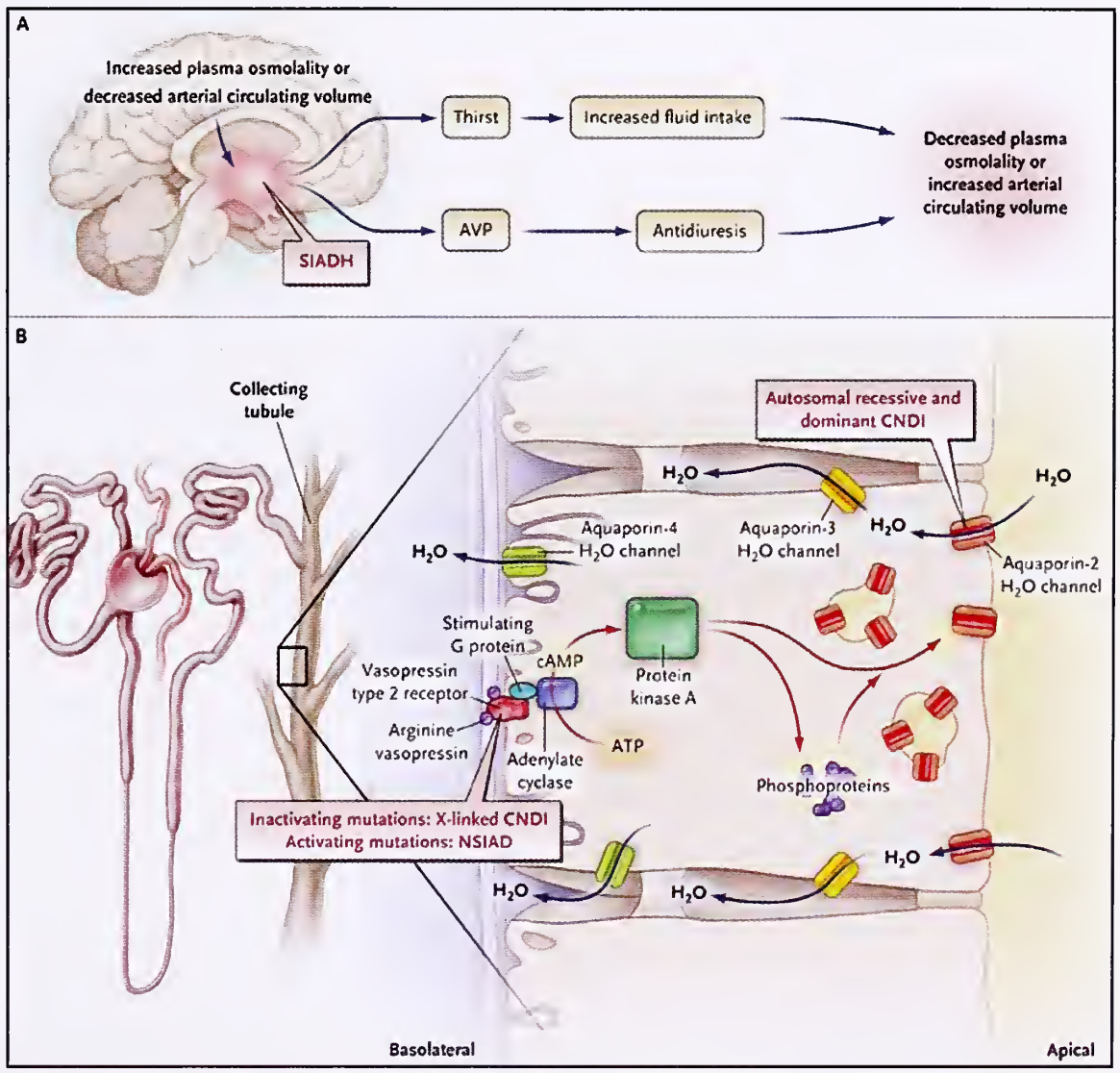
SIADH Due to Gene Mutation in Vasopressin Receptor

The authors described 2 unrelated male infants (2.5 and 3 months of age) with clinical and biochemical evidence of the syndrome of inappropriate antidiuretic hormone secretion (SIADH) including irritability and/or seizures, hyponatremia, hypochloremia, and hypo-osmolality, with relatively increased urinary osmolalities and sodium concentrations. However, instead of measurable values of plasma ADH, concentrations of ADH were undetectable in both infants. In the absence of other causes of SIADH (neural insults, drug exposure), the investigators questioned the possibility of a constitutively active mutation in the gene (*AVPR2*, chromosome Xq28, OMIM 304800) encoding the vasopressin-2 receptor (V2R), a G-protein-coupled 7 transmembrane receptor (GPCR) that activates adenylyl cyclase. Sequencing of *AVPR2* revealed hemizygous mutations in codon 137 (arginine) in both subjects: C770T resulting in Arg137Cys; G771T leading to Arg137Leu. Codon 137 is located in the second intracytoplasmic loop at the end of transmembrane domain III, a highly conserved region in all GPCRs. One of the asymptomatic mothers was heterozygous for the same mutation present in her son; the mutation in *AVPR2* in the other patient was apparently spontaneous. Expression of the mutated V2R in COS-7 cells revealed that basal levels of cAMP generation were 4- to 7-fold greater than that of cells



Physiology of Water Homeostasis in Humans (Panel A) and Pathway of AVP Signaling in Renal Collecting-Duct Cells Involved in Regulating Water Excretion (Panel B). Reprinted with permission Knoers NVAM. *N Engl J Med*. 2005;352:1847–1850. Copyright ©2005. Massachusetts Medical Society. All rights reserved.

expressing wild-type receptor, consistent with a constitutively active V2R. The patients were treated successfully with oral urea to induce an osmotic diuresis. The authors suggested that the possibility of a gain-of-function (GOF) mutation in *AVPR2* be considered in other patients without an apparent cause of SIADH and with low serum concentrations of ADH.

Feldman BJ, Rosenthal SM, Vargas GA, et al. Nephrogenic syndrome of inappropriate antidiuresis. *N Engl J Med*. 2005;352:1884–1890.

Editor's Comment: *The V2R now joins a number of other GPCRs with germline mutations that render them constitutively active and lead to dysfunction of the endocrine system including: LHR–familial male-limited isosexual precocity; TSHR–autosomal dominant nonimmune hyperthyroidism; CaSR–autosomal dominant hypocalcemia; MC2R–corticotropin independent hyperadrenocorticism; FSHR–familial ovarian hyperstimulation syndrome; PTH/PTHrPR–Jansen's metaphyseal chondrodysplasia with hypercalcemia.*[1] *It is of great interest that in patients with nephrogenic diabetes insipidus, the substitution of histidine for arginine at codon 137 has been identified. Thus, different mutations of the same amino acid in V2R lead to functional or non-functional states, emphasizing the critical importance of this site and its effect on the receptor's 3-dimensional configuration. It would also be of interest to assess water homeostatic mechanisms (water loading or deprivation) in the woman who is heterozygous for the GOF mutation in V2R to determine whether there is a gene dosage affect for this protein (Figure).*

Allen W. Root, MD

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JNK vs Insulin/IGF Signaling: Mediating the Effects of Stress and Nutrition on Longevity

Insulin/IGF signaling (IIS) promotes growth and energy storage when nutrients are abundant, but the life span of different eukaryotic organisms (mice, *Drosophila, C. elegans*) is actually increased when IIS is reduced by calorie restriction or by mutations in its pathway components. It seems that resistance to oxidative stress underlies this paradox. Environmental insults such as UV irradiation and oxidative stress activate, among other molecules, Jun-N-terminal kinase (JNK), a component of mitogen-activated protein kinase (MAPK) cascade, that induces a protective gene expression profile and thereby confers tolerance to oxidative stress and prolongs life span.

Wang and colleagues studied the opposing effects of IIS and JNK on oxidative stress tolerance and longevity in *Drosophila*. The *Drosophila* genome contains 7 insulin-like peptides, of which *dilp2* most closely resembles human insulin. *Dilp2* is secreted by insulin-producing cells (IPC) that form a small cluster of neuroendocrine cells in the fly brain. Specific elimination of IPCs leads to growth retardation, developmental delays, and decreased late-life mortality. Similar to humans, *Drosophila* IIS leads to activation of PI3 kinase and Akt, which in turn phosphorylates the Forkhead transcription factor, causing its cytoplasmic retention and down-regulation of its target genes. The *Drosophila* Forkhead transcription factor *DFoxo* extends lifespan when over-expressed.

The authors provided evidence that *DFoxo* is required for JNK-mediated life span extension in *Drosophila*. Further, JNK signaling affected *DFoxo* function as shown by modulation of *DFoxo*-dependent phenotypes in eye development and expression of the *DFoxo* target genes *thor* (a translational repressor that suppresses growth when IIS is inactive) and *l(2)efl* (a small heat shock protein that enhances survival of cells exposed to oxidative damage). Finally, the authors found that JNK and *DFoxo* restrict IIS systemically by repressing *dilp2* expression in IPCs. When JNK activity was specifically increased in the IPCs only, there was a *DFoxo*-dependent decrease in body size and increase in life span. Thus, the JNK-*DFoxo* effects on aging and lifespan occur at 2 levels. In peripheral tissues, JNK activates *DFoxo* to prevent senescence cell-autonomously through expression of genes protective against oxidative damage (eg, preventing age-related declines in cardiac or neurologic function). JNK activation of *DFoxo* in IPCs represses *dilp2* expression, thereby decreasing IIS systemically and coordinating cellular responses to environmental changes, which impacts the life span of the organism as a whole.

Wang MC, Bohmann D, Jasper H. JNK extends life span and limits growth by antagonizing cellular and organism-wide responses to insulin signaling. *Cell*. 2005;121:115–125.

Editor's Comment: *In this intriguing paper, the authors made a strong case for Foxo being the convergence point of the opposing effects of IIS and JNK activity on longevity and stress response in Drosophila. They speculated whether Foxo homologs may play a similar role in mammals, and they cited prior evidence that JNK can inhibit IIS by phosphorylating and inhibiting the insulin receptor substrate.*[1,2]

Another unmentioned convergence point in mammals is the tumor suppressor, p53. Inactive JNK binds the N-terminus of p53, leading to p53 ubiquitination and degradation; this is one of the principal mechanisms by which the tumor suppressor is kept at very low concentrations under normal circumstances. However, when JNK is activated by radiation or oxidative stress, it phosphorylates p53 on threonine 81, thereby activating it.[3] *By repressing transcription of IGF-II and the IGF receptor while activating transcription of IGF binding protein (IGFBP)-3,*

p53 directly inhibits IGF signaling.[4] As well, p53 has been implicated in issues of senescence, longevity, and responses to nutrition and stress. Mice harboring a carboxy-terminus p53 fragment that augments activity of the wild-type p53 allele displayed enhanced resistance to spontaneous tumors, but early onset of aging phenotypes, including osteoporosis, lordokyphosis, generalized organ atrophy, decreased stress tolerance and reduced longevity.[5]

Adda Grimberg, MD

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Congenital ACTH Deficiency, Hypoglycemia and TPIT Gene Mutations

Over the past decades congenital pituitary hormone deficiencies have been described in humans; corresponding animal models were also developed. Quite a number of transcription factor mutations have been reported. Of these, TPIT is the most cell-restricted transcription factor controlling the terminal differentiation of the corticotrophs. Mutations of the *TPIT* gene in humans are associated with congenital ACTH deficiency.

This is the first large report of a neonatal-onset form of congenital isolated ACTH deficiency (IAD). The authors described a series of patients (n=27) from 21 unrelated families. *TPIT* gene mutations, all of which affected coding sequences, were found in only 17 of the 27 patients. Ten different mutations were identified and their distribution indicated a recessive mode of transmission. It was also shown by functional studies of 4 missense mutations that there was a defect in the transcriptional ability with loss of DNA binding, a mechanism inducing a loss of function. The 10 remaining cases belonged to 8 different families who were consanguineous or had evidence of hereditary transmission of IAD.

In the group carrying *TPIT* gene mutations the diagnosis was made before the age of 2 years. Severe hypoglycemia led to the diagnosis of IAD. Furthermore, 11 out of 17 neonates presented prolonged neonatal cholestatic jaundice. These symptoms were suppressed by cortisol replacement therapy. Adrenarche did not occur at time of puberty. In patients without mutations the clinical picture was the same, however, there were some cases with milder disease who had evidence of some ACTH secretion, but it was insufficient to avoid hypoglycemia.

Therefore, congenital IAD, regardless of the molecular findings, presented with a homogeneous clinical phenotype. Consanguinity was observed in 5 of 13 families. Compound heterozygotes were also present, indicating that mutant alleles may be more frequent than expected in the population. It was concluded that the subgroup of IAD patients without mutations should be further investigated for loss-of-function of other genes.

Vallette-Kasic S, Brue T, Pulichino AM, et al. Congenital isolated adrenocorticotrophin deficiency: An underestimated cause of neonatal death, explained by TPIT gene mutations. *J Clin Endocrinol Metab*. 2005;90:1323–1331.

Editor's Comment: *This group, led by Drouin, presented their first paper in 2001 on TPIT, a pituitary cell restricted T-box factor, showing that mutations in this gene were associated with neonatal IAD.[1,2] These researchers now report that a large number of patients and families (some followed-up until puberty) showed lack of adrenarche. The presenting symptoms are characteristic of profound neonatal cortisol deficiency combining hypoglycemia and cholestatic jaundice. Thus, this entity should be considered in the array of causes of early adrenal insufficiency and be considered a neonatal emergency, easily controlled by cortisol treatment. The more puzzling issue is the group of patients who have no mutations in the coding sequence of the TPIT gene. However, their clinical presentation and management were not different.*

Another issue is the severity of hypoglycemia causing neonatal death or mental retardation in survivors. Death occurred in 5 infants belonging to 5 families regardless of the presence of TPIT mutations. Therefore, congenital ACTH deficiency should be rapidly recognized. In affected families prenatal diagnosis should be performed, as in other genetic diseases with adrenal hypoplasia, by measuring maternal serum estriol levels during the third trimester of pregnancy.

In addition, it is of interest that a late onset form of IAD has been described with a presentation of cortisol deficiency without skin hyperpigmentation during childhood.[3] In these cases, mutation of the TPIT gene could not be found. Here again, other genes contributing to this lineage differentiation and to ACTH secretion may be involved. We do not know whether these cases are somehow related to the early congenital form without identified mutations.

Raphaël Rappaport, MD

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Neuropsychological Sequelae and Brain Function in Adults with Childhood-Onset Growth Hormone Deficiency

The researchers set out to further examine reports of cognitive dysfunction in adults with childhood-onset growth hormone deficiency (GHD) and to investigate potential causes in atypical brain metabolism. Eleven adults (7 male and 4 female) with childhood-onset GHD, who had been treated with GH during childhood for 4 to 16 years (mean duration 8.2 years), were evaluated by neuropsychological testing and magnetic resonance spectroscopy (MRS) at least 3 months after discontinuation of GH replacement. The GHD participants were compared to a health- and demographically-matched control group (n=9). MRS was used to assess brain *N*-acetylaspartate (NAA) and NAA/choline ratios, indices of hormonal density and integrity. The GHD group exhibited significantly lower performance on a delayed memory recall task (15-word delayed recall score), a measure of planning behavior, cognitive processing speed, and attention (Trail-making test, Part A). The GHD group also showed significantly lower NAA and NAA/choline levels, and increased choline levels compared to controls. Finally, IGF-I was significantly correlated with NAA levels, but not with choline levels or NAA/choline ratios. The investigators interpret their findings as corroboration of other reports indicating subtle neurocognitive deficits in adults with childhood-onset GHD. Moreover, these effects (in combination with evidence of reduced NAA level in the brain) resemble those observed in normal aging.

van Dam PS, de Winter CF, de Vries R, et al. *Psychoneuroendocrinology*. 2005;30:357–363.

Editor's Comment: *Cognitive function in children and adults with childhood-onset GHD has been the topic of multiple studies. Neuropsychological testing corroborates clinical impressions that associations between GHD and deficits in cognitive performance are subtle; the report by van Dam et al demonstrates an altered brain metabolism while they were off GH treatment. Nevertheless, there is evidence that GHD, which can be a consequence of perinatal insult, cancer (and its treatment), and other pathologic states, may be associated with substantially increased rates of learning disabilities.*[1]

Future studies of this topic will benefit from larger sample sizes and statistical analyses that adjust for gender, participant's global intelligence, and adequacy of hormone replacement in adulthood for those with multiple pituitary hormone deficits. Presently, the benefits of GH replacement in adulthood on cognitive performance remain unclear. Whereas, physiologic doses of GH in individuals with adult-onset GHD appear to be ineffective,[2] *more promising findings derive from a study in childhood-onset GHD.*[3]

David E. Sandberg, PhD

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GROWTH HORMONE AS A THERAPEUTIC AGENT

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Twenty years have passed since recombinant human growth hormone (rhGH) was approved by the FDA for clinical use in patients with growth hormone deficiency (GHD). This was a major breakthrough, as the only previous source of GH was naturally-occurring GH extracted and purified, to a variable extent from human pituitaries removed at autopsy. This human GH (hGH) was first prepared and studied by Raben[1] in 1958 and was shown to produce growth in a sexually undeveloped adolescent. The supply of hGH for investigation and/or therapy was very limited until rhGH became available in 1985, when the supply suddenly became unlimited and the new modern era of GH as a therapeutic agent began. Genentech developed the recombinant techniques to synthesize rhGH, and also developed the necessary testing leading to approval by the FDA of rhGH for human use.

Highlights In This Issue

E-Abstracts (Abstracts On-line)

From The Editor's Desk

This issue's lead article commemorates the 50 years of human growth hormone (hGH) as a therapeutic agent and the 20th anniversary of recombinant hGH (rhGH) for the treatment of hypopituitary children. The personal recollections of Dr. Robert Blizzard bring to the reader a clear historical perspective of the developments that brought about the rise and fall of hGH. It also highlights the synthesis and approval of rhGH and the major strides made with the unlimited availability of rhGH.

We also commemorate 2 decades of *Growth, Genetics & Hormones (GGH)*. This journal was established in 1985 to provide a high-quality educational resource to physicians. The journal accomplished its mission, and more. Dr. Blizzard's leadership, the hard work of the Editorial Board, and an unrestricted educational grant from Genentech made it all possible. In 2002 www.GGHjournal.com was launched. This enabled us to bring *GGH* to most pediatric endocrinologists around the world. From their comments, we know they treasure the content and erudite comments of the Editorial Board. The on line archives of the journal constitute the repository of the fundamental advances that have occurred in the field of growth since the beginning of *GGH*.

Each year we have given readers more material and added features without an increase in budget. However, *GGH* may cease publication next year as the educational grant that we have enjoyed since its inception will not be available after April 2006. Thus, we are searching for sponsorship and have requested grant support from all manufacturers of rhGH. The pharmaceutical companies that compete for market share have a common responsibility to provide high quality educational resources to physicians who prescribe rhGH. I challenge them to promptly fill the void so we may continue bringing state-of-the-art, unbiased, valuable information in the field of growth to our colleagues worldwide. It has been estimated that the annual sales of rhGH are $1.5-$2 ***billion***; 30% of the sales being for FDA approved indications to treat children and adults (Perls TT, Reisman NR, Olshansky SJ. *JAMA*. 2005;294:2086-2090.) Thus, there *must* be funds available to be allocated for the continuation of *GGH*, a highly regarded educational journal.

We will continue to explore sources of support to enable us to provide you with *GGH* on a complementary basis—as it has been done since 1985. On line subscribers recently received a survey to evaluate their interest in helping shape the future of the journal. I am gratified by their response; more than 40% indicated a willingness to pay for a subscription to the journal. I urge all of you to complete the one question survey (www.GGHjournal.com) or to send me a note indicating your interest in a paid subscription (editor@GGHjournal.com) so we may plan the future of *GGH*.

Respectfully,
Fima Lifshitz, MD

Growth, Genetics & Hormones is supported by an unrestricted educational grant from **Genentech, Inc.**

The 20th anniversary of FDA approval of rhGH occurs simultaneously with the 20th anniversary of the establishment of the journal, *Growth, Genetics & Hormones* (*GGH*, available at www.GGHjournal.com). *GGH* has been supported by Genentech, Inc., via an educational grant. *GGH* was the first journal established for the purpose of assimilating published information, both domestic and international, on growth problems valuable to the pediatric community (endocrinologists, geneticists, matabolists, and generalists). The current editor-in-chief of *GGH* believes that a review of the historical aspects of the development and use of hGH and rhGH should be presented during this simultaneously occurring 20th anniversary before the details are lost in obscurity.

I undertake this task as one who has been privileged to be an observer and participant in the accomplishments brought about by Genentech in creating both rhGH and *GGH*. As stated in the first issue, "*GGH* is established as an educational journal by the Editorial Board to facilitate the flow of information and commentary which provide a close look at current, and often controversial, topics in endocrinology, metabolism, and genetics, and their potential applications."[2] This goal has been, and continues to be met, for 20 years. Similarly, the creation and production of rhGH have benefited many thousands of children with growth disturbances. I also undertake this task as one participating actively in the use of native GH in the 30 years preceding the launching of rhGH and *GGH*.

This review is a personal perspective and recall of the past 50 years. In that sense, it may not always be totally accurate and it does not cover all important aspects in the field. Furthermore, these historical comments are made pertaining to my own experience in the United States and, therefore, do not reflect the equally interesting experiences in Europe, South America, Australia, New Zealand, and elsewhere.

VERY EARLY HISTORICAL PERSPECTIVES (Prior to 1958)

The first human who received GH of any origin was a 3½-year-old patient with presumed GHD to whom I gave bovine GH (BGH) in 1956 (supplied by Choh H. Li). This patient received BGH daily over a 3-week period while 24-hour metabolic balance studies were performed. I personally handled all stool, urinary, and dietary samples, and performed appropriate nitrogen and calcium determinations. Neither positive nitrogen balance nor hypercalcuria markers of GH reactivity were demonstrable. The conclusion was that either BGH did not act in humans or the patient was GH insensitive. Later, in 1963 when the immunoassay for hGH became available, high levels of GH and low levels of somatomedin or insulin-like growth-factor-I (IGF-I) were found in this patient's serum.[3] At 10 years of age, the patient did not respond to hGH. Thus, this was the first patient to be diagnosed with GH insensitivity (GHI), eventually named "Laron's Syndrome." Now at 53 years of age, she survives without hypoglycemia (post-pancreatectomy), is married, and has a normal-size son.

EARLY HISTORICAL PERSPECTIVES (1958-1965)

Prior to 1958 studies with GH were pursued primarily in rodents and lower mammals, chiefly in 3 laboratories (led by Choh H. Li, PhD, UC Berkeley; Alfred Wilhelmi, PhD, Emory University; and Maurice Raben, MD, Tufts University). By 1958, each utilized different extraction methods to retrieve hGH from human pituitaries. For example, Raben's procedure used hot glacial acetic acid which destroyed TSH, LH, and FSH. Li's method was the most elaborate as he strived to report the chemical structure of hGH, which he did twice (once incorrectly and subsequently, correctly). Wilhelmi's procedure produced a wide array of pituitary hormones in side fractions, which could be purified and used for clinical investigation.

Initially, the collection of human pituitaries was a diverse effort. Each of the above-mentioned extractors, many other endocrinologists, and even parents of short children solicited pathologists to collect pituitaries on all autopsied patients. Pituitaries from most unembalmed and all embalmed bodies at autopsy were placed separately in acetone, and a majority of those from unembalmed bodies were frozen *en mass*. The latter yielded greater amounts of hormone and the GH was less antigenic. Individual collection programs rapidly developed, usually under the leadership of an individual pediatric endocrinologist or a university group of pediatric endocrinologists. These programs tended to be geographically proximal to the location of one of the extractors. By 1962, Raben was receiving approximately 15 000 pituitaries per year, Wilhelmi was extracting approximately 3500, and Li a few less. Approximately half of the hGH extracted was kept for the extractor's scientific use and the other half was returned to pediatric endocrinologists for clinical investigation of their patients. By 1959, I and a few others were studying presumed GHD patients with native hGH collected and extracted by these methods.

Initially, about 1 mg of hGH was obtained per unembalmed pituitary. Since 1 mg of hGH was needed to treat one patient per day, 365 pituitaries were needed per patient per year. From 20 000 pituitaries extracted per year, about 10 000 mg were available for pediatric endocrinologists. Thus, only 30 patients could receive a full course of therapy. The fascinating story of the collection of pituitaries, for extraction of hGH initially and other hormones subsequently, is a tale of intrigue and secrecy. A black-market competition for pituitaries developed. Scientific collegiality and secrecy occurred simultaneously. Clinical investigation produced many successes and too many disappointments.

In 1961, The National Institutes of Health (NIH) asked me to establish the National Pituitary Agency (NPA) to collect pituitaries on a national basis to counter the ever-growing black market for pituitaries, and to nationally organize and guide the collection, extraction, and distribution of hGH initially and other hormones later. To sell the concept of establishing the NPA was no easy task. Understandably, the extractors and involved pediatric endocrinologists had concerns about collection turfs. After extensive discussions and persuasion, an agreement of extractors, endocrinologists, and pathologists was finally attained. Each participant would be entitled to receive the same amount of pituitaries and/or hGH as he/she had received the previous year. The National Institute of Arthritis and Metabolic Diseases (NIAMD) entered into a contractual agreement with The Johns Hopkins University (my base of operation) to support the necessary personnel (other than myself), office expenses, and payments to pathologists of $2 for the services rendered to collect, store, and deliver each pituitary to the NPA.

Funding for this agency was not available until 1963 (approximately 2 years later). Thus, I had to locate funding from other sources to implement the program. The initial success was due to many dedicated persons including Alfred Wilhelmi, PhD; William Daughaday, MD; Eugene Latimer, MD, physician coordinator; Ms. Dorothy Miller, executive secretary; and many others. The NPA was assisted by parents such as Fred and Gwen Mahler, who had 2 children with genetic GHD. Mr. Mahler, a TWA pilot, arranged transporting frozen pituitaries in the cockpits of planes from major cities in the US to the NPA in Baltimore. Mrs. Mahler, a retired TWA flight attendant, organized other retired TWA flight attendants on a national basis ("TWA Clipped Wings") to raise and donate thousands of dollars annually, for at least 6 years, to fund expenses of the NPA and the Human Growth Foundation which was created by parents of children with growth disturbances.

Of interest are the very crude methods (by today's standards) utilized for the collection and handling of the pituitaries and extracted GH. The hGH was received from the extractors at the NPA in small mason jars. It was transferred by a spatula to wax paper and placed on a simple analytical balance. One mg of hGH was weighed and placed in a small sterile screw cap vial which then was sealed. Multiple vials were then transferred via parcel to the physician investigators, along with 5- or 10-mL vials of various solvents, depending upon which hormone was dispensed. The most disagreeable solvent was 0.1% HCl, which was necessary to use in order for the Raben hGH to go into solution. Patients much preferred hGH from sources other than Raben.

In those early days, no bio-potency was determined and hGH was dispensed and injected on a milligram weight basis. Not until 1965 were potency estimates utilized. Subsequently, assays utilized the growth rate of the tails of rats injected with hGH. The concentrations between batches varied from 0.5 to 2.0 units/mg of hGH. Reading the literature of that period is confusing since often only the milligram designation was used. The amount of hGH extracted per pituitary steadily improved. By 1977 when Albert Parlow, MD, became the single extractor of all human pituitaries in the US, the amount of hGH obtained per pituitary was several times greater than that obtained in 1960. Because of Parlow's efforts the supplies of hGH greatly increased. Remarkably, the hGH distributed never led to infections or adverse reactions until the occurrence in 1985 of the first case of Creutzfeld-Jacob disease (CJD) resulting from the injection of apparently prion-contaminated hGH given many years earlier.

The treatment of patients was on the basis of investigation proposed by clinical research protocols on grant applications submitted to the NPA. Board review was the mechanism used to assess the proposals and to fairly distribute the extracted hGH. By law, the NIH could not support clinical treatment but could support investigative therapy. By 1963, substantial investigative therapy had been accomplished. An Editorial Commentary[4] in 1963 by myself stated that: (1) hGH had been proven to be effective for periods up to 5 years, (2) in the first few months of therapy linear growth accelerated 6 or 7 times the pretreatment period, (3) the effectiveness of the hormone gradually waned, (4) there were no significant side effects detected, and (5) the dosage and schedules in therapy varied widely, but approximately 300 to 500 mg of hGH were required per year for each child treated. Therefore, widespread use was not possible even if a pituitary from each autopsy performed in the US was collected, as even this would only permit therapy in about 4000 patients. The editorial comment also stated that there was reason to believe that the short stature of Turner syndrome and other types of short stature were amenable to therapy. This fact was confirmed several years later. Also stated was the prediction that when hGH would become available in sufficient quantities it would have a breadth of application approaching that of cortisone.

HISTORICAL PERSPECTIVES (1965-1975)

In 1965, a Ross research conference on hGH was held at The Johns Hopkins Hospital, Baltimore, Maryland. The proceedings[5] summarized the state of knowledge at the time, including that in 1962 a radioimmune assay for hGH was published,[6] which permitted insight into GH's action in relation to diagnosis and treatment. By 1966, Alfred Wilhelmi, PhD; Robert Ryan, MD, Mayo Clinic; and Brij Saxena, PhD, Cornell University Medical College, were extracting and purifying TSH, ACTH, LH, and FSH from pituitaries. This ultimately permitted immunoassays for each of these hormones to be developed. It was possible, therefore, to significantly extend investigation of normal and abnormal endocrine physiology, and the

interrelations of hormones of the pituitary, the gonads, and the adrenals at adolescence. In the late 1960s, the development of a constant withdrawal pump by Avinoam Kowarski, MD and his collaborators[7] made it possible to measure integrated concentrations of hGH over various periods of time, which advanced the capability to better understand GH physiology and production in relation to age, gender, and the effect of sex steroids.

The success of collection of pituitaries for hGH therapy, and the accumulation of knowledge derived from the use of hGH, was not without disappointments. In 1965, in a major US city the press learned that pituitaries were being collected by the medical examiner's office and shipped to the NPA. The diener was being paid the customary fee of $2/pituitary for collecting, storing, and shipping the pituitary glands. However, he also collected gold from the mouths of autopsied corpses, and used the money gained from his supplemented income to build a swimming pool in his backyard called "the pit." The news transmitted by the United Press International and Associate Press did its damage. Grand Jury hearings were held in several cities, which affected the number of pituitaries collected that year. Unfortunately, there were other questionable occurrences in conjunction with the NPA's collection. One example involved an employee of the agency who executed questionable transactions for personal benefit. The tasks of the Director and the Board of Directors were not dull and were time-consuming.

GENE SPLICING AND RECOMBINANT DNA (rhGH) (1976-1985)

Based upon the laboratory demonstration that genes could be manipulated to produce useful new substances such as rhGH and rh-insulin, a remarkable story of a scientific revolution unfolded. This manipulation relied upon a controversial new area of research known as recombinant DNA engineering or, more popularly, as gene splicing. Stephen Hall has told the fascinating stories of the race to identify and duplicate the structures of genes (ie, insulin, GH, and somatostatin), the incorporation of these into bacteria, and by 1985 the production of these hormones in mass quantities. His book, *Invisible Frontiers*[8] (Oxford University Press, New York, NY, 1987) is a "must read" for anyone interested in this field. Hall describes the molecular biology which challenged the accomplishment of making these hormones available as therapeutic agents, as well as the personal and professional interrelationships between the scientists. The result is a remarkable documentary of the multiple facets which transected medical science, therapeutic treatment, the pharmaceutical industry, and medical ethics into an entire new world in a 10-year period.

The mass production of specific hormones such as rhGH required identification of the gene structure of the desired hormone, duplicating that structure, determining a way to mass produce the gene, splice the human gene into the gene structure of a bacteria so that the bacteria would produce the desired hormone in large quantity, purify the hormone, test the potency and possible toxicity in non-human mammals, and then test the hormone's potency, effectiveness, and possible toxicity in humans. The concept to accomplish this was clear prior to 1975, but the competitive race to develop the methodology began by scientists in 1976 when 3 groups of scientists in the US started the race to make insulin by recombinant technology. These groups were located at Harvard (Walter Gilbert, PhD, lab chief), at University of California at San Francisco (William Rutter, PhD; Howard Goodman, PhD; and Herbert Boyer, PhD, lab chiefs), and at Genentech (Herbert Boyer). Robert Swanson was a venture capitalist who, with Boyer, had a business goal, specifically, to make and sell human insulin. In August 1978, the Genentech group succeeded. The product was sold to Lilly and operating capital for further projects was available to the Genentech scientists. Peter Seeburg, PhD, a post-doctorate fellow with Goodman at UCSF, had been working with the hGH gene splicing system and joined the Genentech group that subsequently produced rhGH.

By 1981, several pediatric endocrinologists, including myself, were in the process of establishing the protocols for clinical trials of rhGH. A key person and the first physician employed by Genentech for the establishment of the clinical endocrine projects was Ann Johanson, MD, Professor of Pediatrics, University of Virginia. By October 1985, the clinical trials were successfully completed and the FDA approved rhGH for clinical use in patients with GHD.

Serendipity was manifest. In 1985, two explosive occurrences transpired. In March, a patient who had received hGH years previously was reported to have died of CJD. Native hGH had been given to patients for 27 years without significant side effects. The question was asked, "Should hGH investigation and therapy be discontinued?" Mortimer Lipsett, MD, Director of the National Institutes of Diabetes, Digestive and Metabolic Diseases (NIDDM) quickly called a meeting at NIH to discuss the question. Twenty plus prominent physicians of various specialties were present. I led the group who believed that "One is a series of nothing," and my calculation from the data generated by the NPA that 11 miles of height had been given to GHD patients over the years persuaded the consultant group and Dr. Lipsett not to stop distribution. Upon returning home after a follow-up meeting in New Orleans, I found a letter awaiting me from parents of an adult whom I had treated with native hGH as a child. Their son had succumbed to a neuropathological disease several months previously. A third patient also was quickly recognized. The comet truly had exploded and hGH distribution had to be stopped. The second event was the approval of rhGH by the FDA in October of 1985 for

treatment of GHD patients. This was the culmination of a phenomenal development: the creation of a synthetic rhGH that was accompanied by unlimited supplies of hGH for investigation and therapy.

hGH AND rhGH FOLLOW-UP (1985-2005)

This timeframe comprises 2 major areas of interest: first, the follow-up to the use of native hGH during the prior 27-year period (1958-1985), particularly in respect to the status of CJD, and second, the legitimate and illegitimate use of rhGH.

As of January 1, 2003, CJD in the US was reported to have occurred in 26 of the approximately 7700 patients (an incidence of approximately 0.34%) who had received hGH between 1958 and 1985. The names and addresses of 6272 of these are known. The possibility exists that some of the remainder (1428) may have been lost to follow-up because of death from CJD. Distribution of the preparations used in the early years was not always from the same extractor because the NPA often had only one type of preparation to distribute, and by necessity many patients received different preparations while undergoing investigative therapy. By 1977, the Wilhelmi extraction procedure had been dramatically improved in purity and in yield by Parlow, who had assumed responsibility for purification of all hGH for the NPA. No patient started on hGH after this improvement was incorporated into the process has developed CJD. In retrospect, Wilhelmi's preparations most likely were the source of the prion contamination, but even if this is correct only a few of the preparations were probably contaminated. Multiple factors, including total dose of the contaminated preparation and genetic susceptibility undoubtedly affected whether an exposed patient developed the disease. More cases might be expected to be reported, but the pandemic projected by Daniel Gajdusek, MD, PhD, in 1985 never occurred. In April 2003, Allen Spiegel, MD, Director of NIDDK, distributed 2 reports (a comprehensive and a short form) updating the information concerning CJD and hGH. (Information can be obtained on the NIDDK website, www.niddk.nih.gov/health/endo/pubs/cruetz/update.htm).

Other diseases which could possibly be transmitted via hGH—including HIV—have not occurred. Adrenal crisis, however, has allegedly resulted in more deaths in patients having received hGH and rhGH than has CJD. Adrenal crisis is probably not caused by hGH or rhGH, but is a result of associated ACTH deficiency in patients with hypopituitarism. The positive aspects of the follow-up to the use of native hGH are many, and most are known to the readers of *GGH*. Those wishing additional information are referred to multiple articles in the *GGH* archives (www.GGHjournal.com/search.cfm).

In respect to the illegitimate use of rhGH, unequivocally the abuse by athletes is, and should be, of primary concern to society and should be halted. The abuse of prescribing rhGH in an attempt to retard the aging process also should receive attention. My credibility to speak regarding the latter issue is gained from personal experience as I participated in a research protocol as proband (1982–1985) to assess if hGH could *reverse* the aging process. Specifically, I received daily injections of hGH for 2.5 years; 4 other men joined me for the last 2 years.[9] The study terminated in 1985 when CJD was reported in patients who had received hGH. As a result of these early studies and subsequent short-term reports by multiple investigators, I remain unconvinced that hGH can *reverse* the aging process. Unequivocally we should strive to eliminate the abuse of rhGH in attempts to *reverse* the aging process. Unfortunately, the much needed study to determine whether rhGH will *retard* the aging process probably will never be done, as it would require 30 years of rhGH administration to a large group of individuals beginning at the ages of 30-35 years, as well as administration of a placebo to a similar group.

SUMMARY, CONCLUSION, AND COMMENT

This abbreviated history written by my recollection of 50 years of the use of hGH as a therapeutic agent is designed to expose young physicians and others to the use of hGH and rhGH over this extended period. With the exception of Stephen Hall's insightful presentation regarding how recombinant hormones came into existence, I am unaware of any historical accounting of the 50 years of GH. I thank Dr. Fima Lifshitz and the Editorial Board of *GGH* for the opportunity to relate these historical events and to share these with the readers of *GGH*.

In conclusion, now in my golden years, I am grateful to have had the opportunity to know and collaborate with so many giants working in the field of somatotropin investigation in the past, and I continue to meet and learn from the giants working in the field today. I am also grateful, and honored, to have had the opportunity to know and collaborate with my many former fellows and colleagues, all of whom were also my mentors. I cannot possibly record here the names of these wonderful people, but each former fellow and colleague can be assured that I am writing about you. My gratitude is also expended to former and current members of the Editorial Board of *GGH*, all of whom have shared significantly in making *GGH* an outstanding journal in bringing together physicians of multiple specialties to share knowledge of common need. The initial goals of Genentech and myself as first Editor in Chief have been exceeded, and continue to be exceeded beyond expectation.

I also wish to thank my former colleagues at NIH and others for the professional opportunities that have been given to me. To Genentech, this double 20th anniversary of the marketing of rhGH and the establishment of *GGH* is worthy of commemoration. Hopefully, this article

adequately recants the significant accomplishments and value of both.

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ADDENDUM

After submitting the above manuscript I became aware that after the March, 2006 issue (Vol 22, No 1), *Growth, Genetics & Hormones* may no longer have funding and thus cease publication. This journal has accomplished the significant goals set forth 20 years ago to broaden sharing of knowledge across pediatric endocrinology, genetics, metabolism and general pediatrics. Furthermore, the same goals need to be continued, as the there is still a great need for sharing of important knowledge to provide the highest level of patient care and research among geneticists, nephrologists, endocrinologists, gastroenterologists, general pediatricians and others. There is no other journal that fulfills the need. Hopefully Genentech will continue to take the lead as they have in the past in so many endeavors, and either support directly the educational grant or organize collegially collaborative support among other organizations or corporations, so that *GGH* continues to be published next year and thereafter.

ABSTRACTS FROM THE LITERATURE

Idiopathic Short Stature Children Are Poor Eaters and Are Thin

Data on the eating behaviors and nutritional status of children with idiopathic short stature (ISS) are lacking. The paper by Wudy et al assessed 214 patients with ISS from 123 families and recorded the BMI and eating behaviors with the Child Eating Behavior Questionnaire and the Food Frequency Questionnaire. Endocrine markers of body weight regulation (leptin and ghrelin) were also measured. The ISS patients had a decreased BMI (–0.33 SDS) as compared with population norms. Furthermore, they also had a decreased food responsiveness with a score of 1.9 on the Child Eating Behavior Questionnaire, as compared with a score of 2.4 for the population mean. They had reduced enjoyment of food (3.2 vs 3.9), emotional under-eating (2.6 vs 3.0), and showed increased fussiness over food (3.2 vs 2.9). "Poor" eaters showed more marked alterations in BMI and behavioral characteristics than those who were "good" eaters. Total serum ghrelin was not different among good and poor eaters, and serum leptin was moderately increased but did not differ between the groups. The authors concluded that ISS patients present altered eating behaviors that possibly contribute to their short stature.

Wudy SA, Hagemann S, Dempfle A, et al. Children with idiopathic short stature are poor eaters and have decrease body mass index. *Pediatrics*. 2005;116:e52–57.

Editor's Comment: *There are countless papers dealing with ISS and other forms of short stature, but the nutritional status and eating behaviors of the patients are rarely addressed. Indeed, low IGF-I levels are most often analyzed and considered essential for diagnosis and treatment of short stature patients, as well as for the publication of scientific papers, often without addressing body weight, dietary intake, or nutritional status. Thus, I am delighted to note the paper by Wudy and colleagues showing ISS patients presenting with alterations in eating patterns and decreased BMIs. Hopefully, these data will stimulate an interest in evaluating the role of suboptimal nutrition on the growth patterns of children with ISS and other short stature patients. This assessment should be a must before embarking in other more costly medical interventions.*

Fima Lifshitz, MD

Compliance with Medication Recommendations

Compliance is defined as "the extent to which a person's behavior coincides with medical or health advice." Despite the importance of the medication in treatment, disease prevention, and health promotion, compliance rates range from 11% to 93%. The authors reviewed pediatric medication compliance literature based on Medline searches of: medication compliance, patient compliance, patient dropouts, or treatment refusal combined with 45 other terms including drug therapy and specific formulations or methods of drug delivery. Additionally,

data were excerpted from an AAP Periodic Survey on primary care pediatricians' views on patient compliance with completing prescriptions for acute and chronic illness. The authors noted a caveat regarding compliance data reliability: parental reports of compliance have been shown to be markedly overrated (eg, in one study, mothers reported 60% compliance with obtaining prescribed refills, compared to only 12% according to pharmacy records).

The review yielded a number of principles pertaining to barriers to good medical regimen adherence. Limits on the *time* the physician can spend with each patient and family to negotiate a best-fit medication and discuss their ability to comply with the prescribed regimen represents a significant barrier. Lack of *continuity of physician-patient interaction*, particularly between and within multi-personnel office settings, is a strong predictor of poor compliance. *Patient and family characteristics* constitute additional sets of factors influencing compliance: the patient's and family's ability to understand the importance of following the prescribed treatment is an important element. Factors affecting understanding include health literacy, education, and culture. Patient/family knowledge, information, and misinformation or perspectives from outside sources (including the Internet) influence compliance, as can psychological function (eg, psychopathology). Such preexisting or emerging problems necessarily require attention in order to enhance compliance.

Practice setting characteristics and *specific physician behaviors* can influence compliance. Parents are more likely to be actively involved in the communication process if they are not distracted by restless children, their own time constraints, and annoyance over long waits. Enhanced communication skills have been known to shorten visit duration, improve patient adherence, and decrease the need for follow-up care.

Medication factors (eg, duration, schedule, formulation, palatability, cost, and adverse effects) were clearly associated with compliance. Longer *duration* of the medication regimen and increased *complexity* of the medication schedule represented risk factors to adherence, with mid-day dosings being particularly problematic. Personal preferences and aversions became evident in relation to *forms of medication* and *palatability.* Children expressed preferences for one form over another (eg, sprinkles vs syrup) whereas parents preferred oral liquid to solid forms (eg, powder, tablets, capsules). *Medication cost* for the uninsured or under-insured constituted an additional burden leading to compromised compliance. Cost also drove drug formulary decisions that restricted access to some useful medications that were more palatable and/or facilitated the dosing schedule. Finally, *adverse effects from medications* decreased compliance.

The authors outlined a set of *General Principles to Enhance Medication Compliance* that include: (1) improving communication between physician and patient/family, (2) modifying or negotiating regimens, (3) emphasizing patient self-management of disease or illness, (4) using the simplest and most effective regimen available, and (5) using technology and devices to facilitate compliance. The authors stated the overriding issue for improved compliance is developing a one-on-one relationship between "1 doctor and 1 patient."

Winnick S, Lucas DO, Hartman AL, Toll D. How do you improve compliance? *Pediatrics*. 2005;115:e718-724.

First Editor's Comment: *The term compliance is often used interchangeably with adherence, as it has been in this paper. However, compliance entails obedience to a directive from a physician (eg, "take this medication 3 times a day"), whereas adherence implies that the patient and family are active collaborators in the treatment process. The WHO defines adherence as "the extent to which a person's behavior—taking medication, following a diet, and/or executing lifestyle changes, corresponds with agreed recommendations from a health care provider."*[1]

The average adherence to medication recommendations is approximately 50% in the pediatric population.[2] *Despite intuitive expectations, adherence can falter even in life-threatening conditions such as type 1 diabetes (T1DM) and congenital adrenal hyperplasia. Winnick et al emphasized the critical importance of the one-on-one relationship between physician and patient as the key to improving adherence. Improved delivery systems (eg, pumps, transdermal patches, etc.) alone are unlikely to eliminate adherence problems. For example, a good collaborative relationship associated with clear communication would facilitate prompt discovery that the adolescent with uncontrolled T1DM has "broken" insulin pumps because he is embarrassed that his peers can see the device. Another example would be the young adult male with gonadotropin deficiency who fails to adhere to recommendations because the testosterone replacement dose is inadequate for normal erectile function. There is typically an explanation for poor adherence, but the remedy presupposes strong lines of communication between the physician and the patient and the family. The cost is time—the time to develop and maintain a relationship. While technological advances can facilitate adherence, when problems emerge, they cannot be confused with the solution.*

Finally, the authors' recommendation to "emphasize patient self-management of disease or illness" should be interpreted cautiously. In the pediatric context, one needs to know who assumes responsibility for various aspects of medical care or how that responsibility is shared within the family.

David E. Sandberg, PhD

Second Editor's Comment: *Coincidentally, Osterberg and Blaschke*[3] *published a review article, "Adherence to Medication" which denotes the importance of this issue across disciplines. As C. Everett Koop said, "Drugs don't work in patients who don't take them." The problem is of particular importance to pediatric endocrinologists*

who treat patients with chronic conditions requiring long-term therapy, complex regimens, and frequent medication changes. Furthermore, patients are often asymptomatic and cannot care for themselves. These patient characteristics are typical of poor compliance and/or adherence to treatment. Lack of response to medication, missed appointments, presence of psychological problems, and/or cognitive impairment of the patient or caregiver may be indicators of poor adherence. High medication costs and third-party payor requirements including high co-payments and frequent refills compound the problem. These barriers are important and add to the time required to obtain medications. Poor adherence contributes to worsening of disease, increased costs of care, and even death. New cost-efficient technologies that facilitate treatment adherence are needed to aid physicians and patients in meeting the goals of therapy.

Fima Lifshitz, MD

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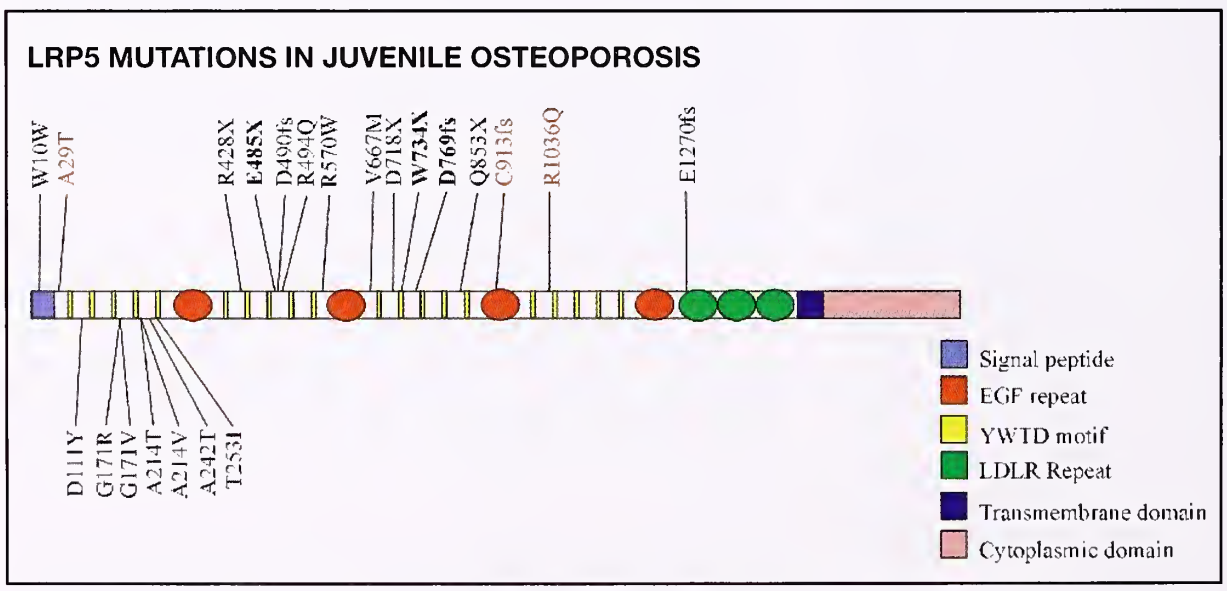
LDL Receptor-Related Protein Mutations in Primary Osteoporosis

The LDL receptor-related protein 5 gene (*LRP5* – OMIM 603506, chromosome 11q13.4) is a 1,615 aa transmembrane protein that interacts with the secreted glycoprotein WNT (wingless – OMIM 604663, chromosome 2q35) and its Frizzled receptor to enhance autocrine WNT signaling of osteoblast-induced bone formation. The interaction of LRP5-WNT-Frizzled receptor is inhibited by another protein termed dickkopf (*DKK* – OMIM 605189, chromosome 10q11.2) that binds to *LRP5* near its amino terminal and interrupts Wnt signaling, thereby modulating the extent of osteogenesis. When a mutation in this region of *LRP5* prevents its binding to DKK, there is further increase in WNT signaling and bone formation leading to high bone mass. Homozygous loss-of-function (LOF) mutations throughout other regions of *LRP5* have been identified in subjects with the osteoporosis-pseudoglioma syndrome (OMIM 259770), an illness characterized by developmental delay, seizures, impaired vision due to a pseudoglioma of the retina, and lax ligaments, as well as decreased bone mineralization.

Hartikka et al found heterozygous LOF mutations in *LRP5* in 3 out of 20 children and adolescents with primary osteoporosis, defined as isolated osteoporosis without stigmata of other illnesses and manifested by fractures with low impact trauma beginning in early childhood. Two missense mutations (Ala29Thr, Arg1036Gln) and one frame shift mutation (Cys913fs) were detected. Examination of family members revealed osteoporosis and similar mutations in a parent and/or a sibling, indicating autosomal dominant transmission of this trait attributable to haploinsufficiency of *LRP5*.

Hartikka H, Mäkitie O, Männikkö M, et al. Heterozygous mutations in the *LDL receptor-related protein 5 (LRP5)* are associated with primary osteoporosis in children. *J Bone Miner Res.* 2005;20:783–789.

Editor's Comment: *Osteopenia and osteoporosis in children and adolescents are most commonly secondary to chronic illnesses, nutritional deprivation, limited mobility, excessive exposure to glucocorticoids, or deficiencies in growth, sex, and/or thyroid hormones. Osteogenesis imperfecta (OI) is due to heterozygous LOF mutations in the genes encoding components of type I collagen (COL1A1, COL1A2). In addition to osseous fragility, patients with OI often have blue sclerae, joint laxity, and dental abnormalities. None of the patients studied by Hartikka et al had a mutation in either of these genes. Juvenile idiopathic osteoporosis*



Schematic representation of the LRP5 protein and its domain structure. Mutations of individuals with OPPG are indicated above the protein structure; heterozygous mutations are marked in bold. Mutations of individuals with high bone mass phenotype, situated in the first YWTD/EDF domain, are indicated below the protein. Mutations of individuals with primary osteoporosis (this study) are marked on red.

develops 2 to 3 years before puberty and is manifested by the acute onset of bone pain due to long bone fracture(s) or vertebral collapse. Heterozygous gain-of-function (GOF) mutations in LRP5 impair binding to DKK and lead to increased bone mass, a trait transmitted in an autosomal dominant manner.[1] Although initially considered a benign variant, later reports associated this trait with intracranial hypertension, cranial nerve palsies, and extensive maxillary and mandibular exotoses.[2] The phenotype of the subject with homozygous GOF mutations in LRP5 has not been described, but might be anticipated to be a lethal form of osteopetrosis.

Allen W. Root, MD

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Aromatase Inhibitor and Growth in the Pubertal Male with GHD

Mauras and colleagues conducted a 12-month pilot study of 20 adolescent males with clinical and biochemical evidence of growth hormone deficiency (GHD) who were treated with GH (mean dose ~0.3/mg/kg/wk) for at least 6 months (range: 6 months–9 years) prior to the study. The investigation sought to determine whether treatment over a period of 12 months with the aromatase inhibitor anastrozole can achieve sustained suppression of estrogen production and delay epiphyseal fusion in adolescent males with GHD. Physical examination, genital Tanner staging, bone age, DEXA scan, and an early morning blood sample were obtained at baseline and throughout the duration of the study. Ten boys were maintained on GH only and 10 were started on anastrozole (1 mg orally daily) in addition to GH.

Results showed a 60% drop in estradiol

Figure 1. Change in Estradiol Concentrations at 12 months

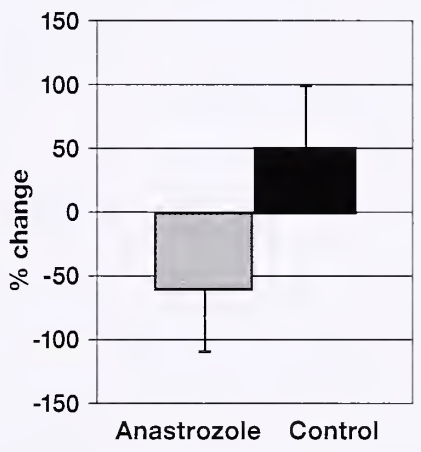

Figure 2. Change in Testosterone Concentrations at 12 months

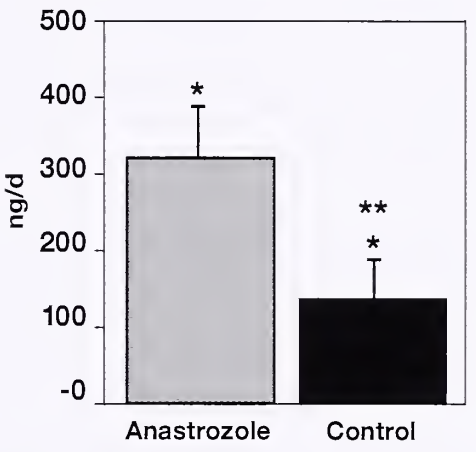

Figure 1. Percentage change in plasma estradiol concentrations.

Figure 2. Absolute change from baseline in serum testosterone concentration. * refers to the difference within each group at 12 mo vs baseline; ** refers to the difference between groups: * p = 0.001; ** p = 0.03.
Adapted from Mauras N, et al. *J Pediatr Endocrinol Metab.* 2004;17:1597-1606. Copyright © 2004. *JPEM*.

Growth, Genetics & Hormones

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concentrations in the anastrozole group and a 50% increase in concentrations in the control group (GH only; Figure 1). The reciprocal increase in testosterone and free testosterone concentrations in the anastrozole group was substantially greater than the rise in testosterone during spontaneous puberty in the control group (Figure 2). IGF-I and IGFBP-3 did not change significantly in the anastrozole group, whereas IGF-I rose significantly at 12 months in the control group. There were no significant differences between the anastrozole and control groups with regard to lipid concentrations, body composition, or bone density, nor any differences in growth velocity rates, height SD scores, bone age advancement, or predicted adult height.

The authors concluded that compared to GH-deficient boys treated with only GH, 12-month treatment with an aromatase inhibitor in combination with GH results in a significant and sustained suppression of circulating estrogen concentrations and reciprocal increases in testosterone concentrations. Anastrozole treatment was not associated with detectable detrimental effects on body composition, tempo of puberty or bone mineralization, and was well tolerated and safe over the period studied. The lack of effect of anastrozole on growth velocity, bone age advancement, or predicted adult height was interpreted by the investigators to be due to the limited duration of use (ie, 12 months).

Mauras N, Welch S, Rini A, Klein KO. An open label 12-month pilot trial on the effects of the aromatase inhibitor anastrozole in growth hormone (GH)-treated GH deficient adolescent boys. *J Pediatr Endocrinol Metab.* 2004;17:1597–1606.

Editor's Comment: *It is reasonable to predict that there will be more studies examining the growth-promoting benefits of aromatase inhibitors. They offer the promise of prolonged growth without the metabolic and psychological drawbacks of arresting pubertal development. This controlled pilot study opens the way to a larger and longer duration study of the synergistic benefits of GH and anastrozole on adult height. Because of the role that sex hormones play in brain development and function,[1] it would be prudent to include neuropsychological endpoints in any study that alters the typical ratios observed between testosterone and estradiol in adolescent males.*

David E. Sandberg, PhD

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COMT Polymorphism in Early Puberty

Estrogens are initially metabolized by a P450 hydroxylase to less biologically active catechol-estrogens and further degraded by catecholamine-O-methyltransferase (COMT) to inactive products. COMT transfers a methyl group from S-adenosylmethionine to the catechol-estrogen. Within the structure of *COMT* (OMIM 116790, chromosome 22q11) is a functional polymorphism that results in the substitution of methionine for valine in codon 158 (val158met). The *COMT* product containing val158 is 3 to 4 fold more biologically active than is that with met158.

Hypothesizing that the COMT val158met polymorphism effect on estrogen catabolism might be reflected in the biologic activity of endogenous estrogen, the investigators examined the relationship between linear growth, bone mineralization, and sexual development in prepubertal and early pubertal, 10-12 year old girls and the high (H) and low (L) polymorphic variants of COMT ($COMT^{HH}$ and $COMT^{LL}$, n=43 and n=85, respectively). Although total serum estradiol concentrations were similar in $COMT^{HH}$ and $COMT^{LL}$ girls, levels of free estradiol as well as IGF-I were higher in $COMT^{LL}$ subjects. The authors reported that at the time of study $COMT^{LL}$ subjects were 5.4 cm taller than were $COMT^{HH}$ girls, had greater lean body mass, and appeared to progress further into puberty at a more rapid rate than did $COMT^{HH}$ girls. Total bone mineral content (BMC) by DEXA was 12.7% greater in $COMT^{LL}$ than in $COMT^{HH}$ girls due primarily to increased bone size; thus, volumetric bone mineral density was similar among groups. Cortical BMC and cortical cross-sectional area by peripheral quantitative computerized tomography (pQCT) were highest in $COMT^{LL}$ subjects due primarily to increased periosteal circumference. There was no relationship between COMT variant and trabecular volume or mineralization. Serum free estradiol values were related to these varied aspects of growth and mineral metabolism and thus indirectly to the COMT polymorphic variants. The authors concluded that the val158met COMT polymorphism exerted significant effects on growth, pubertal development, and bone mineralization in pre- and early adolescent girls, primarily by increasing serum concentrations of free estradiol by altering the rate of catabolism of endogenous estrogens.

Eriksson A-L, Suuriniemi M, Mahonen A, Cheng S, Ohlsson C. The COMT val158met polymorphism is associated with early pubertal development, height and cortical bone mass in girls. *Pediatric Res* 58:71-77,2005.

Editor's Comment: *COMT may now be added to the growing list of recognized genes that influence the rates of growth and sexual development and bone mineralization; this report emphasized the extensive genetic variability in these processes. Although $COMT^{LL}$ girls were 5 cm taller than $COMT^{HH}$ subjects at 10-12 years of age, the effect of the COMT val158 met polymorphism on adult height is likely to be less impressive as the $COMT^{LL}$ subjects will probably*

complete their pubertal development and achieve their adult height at an earlier age than the COMT^{HH} children. It would be of interest to learn the bone ages of the study subjects and later their ages of menarche and their adult heights. Similar studies relating COMT genotype and growth, pubertal maturation, and bone mineralization would also be of interest.

Allen W. Root, MD

Novel Deletions Downstream of *SHOX* Cause Léri-Weill Dyschondrosteosis

Léri-Weill dyschondrosteosis (LWD, MIM 127300) is a dominantly inherited bone dysplasia characterized by short stature, mesomelic limb shortening, and Madelung deformity of the forearm. Heterozygous deletions of the short stature homeobox-containing gene (*SHOX*) occur in LWD, and homozygosity for such deletions has been found in the more severe Langer mesomelic dysplasia (MIM 249700). *SHOX* resides in the pseudoautosomal region 1 (PAR1) of the short arm of the X and Y chromosomes. Its product is involved in cell cycle and growth regulation; and loss of *SHOX* function has also been implicated in Turner syndrome and in some cases of idiopathic short stature.

Detection of *SHOX* defects in only about 60% of LWD patients has raised the possibility that some of the remaining patients could have mutations in regulatory elements that lie upstream or downstream of the *SHOX* locus. Indeed, Benito-Sanz and colleagues have shown this to be the case.

The authors screened 80 LWD patients in whom mutation in the *SHOX* coding sequence had been excluded. Novel deletions in PAR1 downstream of *SHOX* were detected in 12 patients, 8 of whom came from families showing dominant inheritance of LWD. Deletion mapping revealed that the deletions were ~ 30–205 kb downstream of *SHOX* and varied in size from <81 to ~ 501 kb. Fine mapping disclosed a minimal commonly deleted region of 29 kb. The 5' end of the deletion was similar in 5 families, suggesting the possibility of a hotspot for a deletion breakpoint.

Large-scale deletions were detected in 4 families, raising the possibility of a chromosomal rearrangement. However, inversion or translocation of the deleted region to an autosome was excluded by fluorescent in situ hybridization analysis, showing that the deletions were contiguous in all 4 cases.

Two explanations were considered. The first was the presence of a nearby second gene in PAR1 that influences skeletal growth much like *SHOX*. The authors found no evidence for a second gene. The second hypothesis, which was favored by the authors, was that a positive regulatory element for *SHOX* resides in the deleted region; deletion of this region would be expected to produce loss of *SHOX* expression. They noted other examples of long-distance gene regulators, especially involving transcription factors during development.

Benito-Sanz S, Thomas NS, Huber C, et al. A novel class of pseudoautosomal region 1 deletions downstream of *SHOX* is associated with Léri-Weill dyschondrosteosis. *Am J Hum Genet.* 2005;77:533–544.

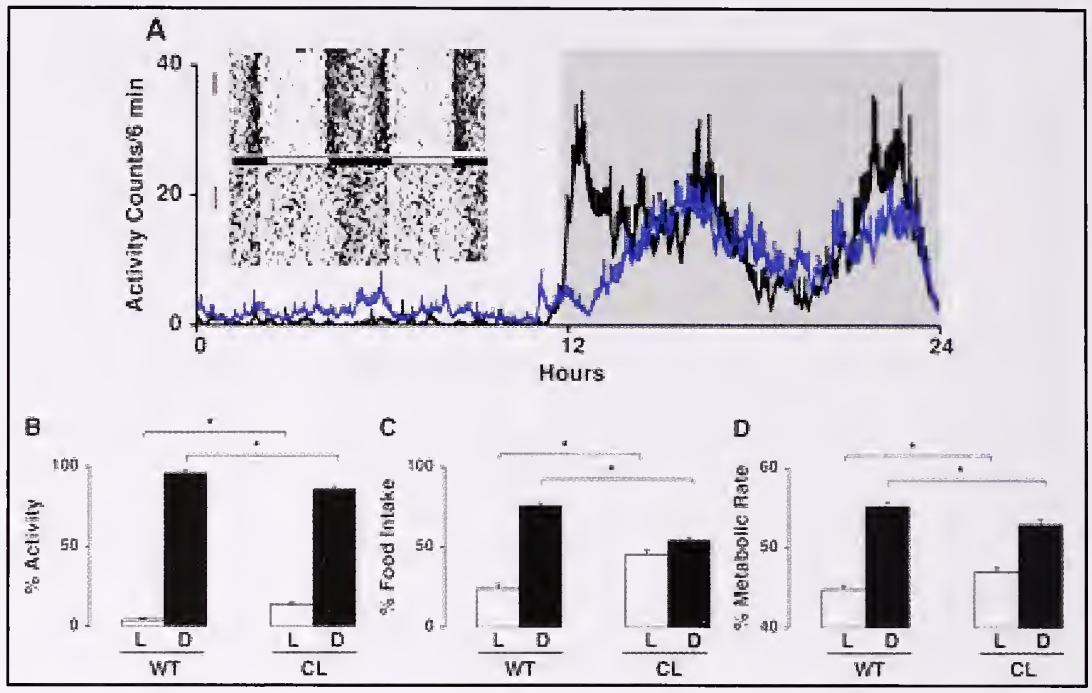
Editor's Comments: *These results help to explain at least some of the 40% of patients in LWD in whom mutations in the SHOX coding sequence are not detected. They underscore a mechanism that is probably under-appreciated: disturbance of long-range gene regulation. It will be interesting to see how common this mechanism will be found to explain similar situations for other genetic diseases.*

William A. Horton, MD

Circadian Rhythms in Obesity and Metabolic Syndrome

Circadian rhythms are governed by a series of regulatory oscillators expressed in the suprachiasmatic nucleus (SCN), and elsewhere in the CNS as well as in most peripheral tissues, that oscillate with an approximate 24-hour periodicity usually entrained to the light-dark cycle.[1] In mice, *Clock* (Circadian Locomotor Output Cycles Kaput - OMIM 601851) encodes an 855 aa transcription factor involved in this process. Overexpression of *Clock* shortens period length. Expression of an A to T nucleotide transversion in a splice donor site that leads to exon skipping and deletion of 51 aa results in 1-hour lengthening of locomotor activity in the heterozygous state and 3- to 4-hour increase in periodicity and dampening of the amplitude of circadian rhythms, leading to loss of periodicity (arrhythmia) in the homozygous animal maintained in constant darkness. Stimulated by the observation that reduced forms of the nicotinamide adenine dinucleotide (NAD) cofactors enhance, and oxidized forms inhibit, DNA binding of the *Clock* transcript, Turek et al investigated the relationship between circadian rhythmicity and intermediary metabolism in homozygous *Clock* mutant mice ($C^{-/-}$) maintained on a 12-hour light-dark cycle. They demonstrated that relative to wild-type (WT) mice, the $C^{-/-}$ mice had decreased locomotor activity during darkness. Also, the $C^{-/-}$ animals ate rather evenly through the 24-hour period, whereas the WT mouse ate 3-fold more during darkness than during light. In addition, the $C^{-/-}$ mice expended 10% less energy per 24 hours than did the WT animals. $C^{-/-}$ animals were heavier than WT animals by 6 weeks

of age; between 6-16 weeks of age, $C^{-/-}$ mice ate greater amounts of food and gained more weight than did WT mice, whether ingesting a normal or high-fat diet. At 7 to 8 months of age, $C^{-/-}$ animals had higher concentrations of leptin, glucose, cholesterol, and triglycerides than did WT mice, but they had similar levels of insulin. Histologically, there were hypertrophy of adipocytes and excessive glycogen and lipid within liver cells (steatosis) in $C^{-/-}$ animals. In the mediobasal hypothalamus, the diurnal patterns of expression (mRNA levels) of orexin and ghrelin (orexigenic agents) and of CART (cocaine- and amphetamine-regulated transcript—an anorexigenic agent) were decreased in $C^{-/-}$ mice relative to WT animals. The authors concluded that *Clock* and the circadian rhythms it controls have regulatory effects on energy intake and expenditure and fuel metabolism. When altered, the resultant abnormalities lead to a syndrome of obesity, hyperglycemia, and hyperlipidemia that mimics the metabolic syndrome and that might be mediated through hypothalamic pathways that regulate appetite and energy utilization.



Altered diurnal rhythms in locomotor activity, feeding, and metabolic rate in *Clock* mutant mice. (*A*) Activity counts over the 24-hour cycle during light (unshaded) and dark (shaded) periods (*B*) Diurnal rhythm of locomotor activity for mice in (A). Total activity over the 24-hour period was similar between wild-type (WT) and *Clock* mutant (CL) genotypes. (*C*) Diurnal rhythm of food intake. Results shown are average food intake during light and dark periods as a percentage of total food intake (*$P < 0.001$). (*D*) Diurnal rhythm of metabolic rate. Results shown are average metabolic rates during the light and dark periods as a percentage of total metabolic rate. All results shown are expressed as group means ± SEM.

Turek FW, Joshu C, Kohsaka A, et al. Obesity and metabolic syndrome in circadian *Clock* mutant mice. *Science*. 2005;308:1043–1045.

Editor's Comment: *Circadian rhythms are present not only in neurons within the SCN but also in single cells in most peripheral tissues and utilize the same regulatory mechanisms found in the SCN.[2] Thus, it is likely that the SCN synchronizes overall diurnal rhythms, while local oscillators regulate tissue-specific circadian function. It is unclear whether the metabolic effects of the described loss-of-function mutation in Clock are exerted through the SCN or in peripheral tissues, but the results of loss of diurnal variability on lipid and carbohydrate metabolism are striking. In volunteer human males, sleep deprivation lowered leptin and increased ghrelin values leading to increase in hunger and appetite.[3] Future studies evaluating the role of the sleep-wake cycle on intermediary metabolism and the genesis of the metabolic syndrome in man are clearly warranted.*

Allen W. Root, MD

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Adult Height in Turner Syndrome

Although it has long been recognized that growth hormone (GH) treatment increases the adult height of those with Turner syndrome, this Canadian study is the first randomized controlled trial carried through to adulthood. Girls with Turner syndrome, aged 7–13 years, were randomly assigned to either receive GH treatment (0.30 mg/kg/wk by subcutaneous injection 6 times per week; n = 76) or to be a part of an untreated control group (C) that did not receive GH treatment (n = 78). Sex hormone replacement was used to induce puberty in both cohorts at age 13 years if onset did not occur spontaneously. Growth hormone treatment lasted on average 5.7 years. Protocol completion required an annualized height velocity of <2 cm/yr and a bone age of 14 years or greater. There were 104 patients (61 GH, 43 C) that completed the protocol (50 withdrew). At protocol completion, mean heights were 147.5 ± 6.1 cm (GH) and 141.0 ± 5.4 cm (C) ($P<0.001$). Girls who

started GH at an earlier age showed a greater increase in adult height (+0.22 SD, 95% CI 0.10–0.33 SD, or +1.5 cm/yr for each year of earlier GH initiation [$P<0.001$]), although this age effect was highly variable between patients. Two follow-up visits further verified the adult height and assessed safety. For those available at least 1 year after protocol completion (n = 59; 40 GH, 19 C), mean heights were 149.0 ± 6.4 (GH) and 142.2 ± 6.6 cm (C) ($P<0.001$). The estimated height gain attributable to GH was +7.2 cm at protocol completion (CI = 6.0–8.4), and +7.3 cm (CI = 5.4–9.2) at follow-up (at least 1 year after protocol completion).

This report was accompanied by an editorial by Carel, in which he chronicled the history of GH treatment for short stature in Turner syndrome. Carel estimated that height gains across studies ranged from "minimal" to 17 cm for high-dose GH treatment. He applauded the Canadian researchers for adopting a powerful research design and carefully following participants. Although he noted that GH treatment unquestionably increased adult height in women with Turner syndrome, he posed a number of provocative questions regarding the cost-effectiveness and safety of GH in this population.

Canadian Growth Hormone Advisory Committee. Impact of growth hormone supplementation on adult height in Turner syndrome: Results of the Canadian randomized controlled trial. *J Clin Endocrinol Metab.* 2005;90:3360–3366.

Carel JC. Editorial: Growth hormone in Turner syndrome: Twenty years after, what can we tell our patients? *J Clin Endocrinol Metab.* 2005;90:3793–3794.

Editor's Comment: *Although some readers may view the study's findings as old news, the accompanying editorial highlights details of its importance. By contrasting these findings with those from a recently published report of a French population-based cohort of GH-treated patients,*[1] *several important questions/observations arise, as succinctly summarized in the Carel editorial. First, insofar as adult height gained (2.7–11.7 cm for those initiating treatment between 7-13 years) varies substantially across patients, most importantly attributable to age at treatment initiation, Carel asks whether GH should be used if only a minor effect is anticipated. Second, Carel claims that adult height gained with GH is not a validated proxy measure for "quality of life" which he identifies as the primary rationale for treatment. In his study, 88% of young adult participants favorably rated their GH treatment. However, when asked to estimate the minimal height gain they thought would make GH treatment worthwhile, the figure was above 8 cm in 64% of cases. Thus, based on adult heights achieved in the French cohort, two-thirds of patients treated with GH in the Canadian trial and many other studies would not consider treatment "worthwhile." Third, findings from this study cannot be extrapolated to adult height outcomes that might be achieved should GH treatment be initiated earlier than 7 years or using higher doses. Finally, Carel focuses on the safety profile of GH treatment. He acknowledges that the overall safety record is good, but cites 2 studies that linked GH therapy with a heightened risk of otitis media. Furthermore, the presence of hearing difficulties in adulthood was found to be a robust predictor of a more negative quality of life in Turner syndrome patients. GH treatment and the associated theoretical risk for cancer was also noted, and careful monitoring of IGF-I levels and long-term follow-up studies were recommended.*

David E. Sandberg, PhD

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Effectiveness of Hydrocortisone and Cortisone Acetate for the Treatment of CAH

Although cortisone acetate (CA) is used worldwide as corticosteroid substitution therapy in congenital adrenal hyperplasia (CAH), its effectiveness is uncertain since CA must be converted to cortisol to be biologically active. Its biologic activity depends on the activation by 11β-hydroxysteroid dehydrogenase (11β-HSD) reductase. Inada et al reported that hydrocortisone (HC) is more effective than CA for the treatment of CAH. The authors compared the effect of CA with that of HC in 10 patients (aged 4–35 years) with 21-hydoxylase deficiency (21-OHD). Of the 10 patients, 8 were salt losers who required fludrocortisone in addition to glucocorticoids. HC was administered to all subjects instead of CA; the initial dose was 80% of the previous CA dose, since the overall bioactivity of oral CA has been reported to be 80% of that of HC. The dose of HC was subsequently changed in accordance with the circulating levels of serum 17-hydroxyprogesterone (17-OHP) and/or plasma adrenocorticotropin (ACTH). Doses of fludrocortisone were

Serum levels of Cortisol, Cortisone, and Cortisol/Cortisone Normal Subjects by Age [1]

Age		Cortisol (ng/ml)	Cortisone (ng/ml)	Cortisol / Cortisone
<2 months	n = 58	29.2 ± 32.6	39.4 ± 21.1	0.9 ± 1.0
≥2 months, <2 years	n = 30	53.7 ± 30.5*	36.3 ± 18.5	1.7 ± 1.0*
≥2 years, ≤20 years	n = 30	71.2 ± 39.1*	24.3 ± 12.3	3.3 ± 1.7*

Data are mean ± SD. *P <0.01 compared with <2 months.

not changed. Target concentrations were below 10 ng/ml for 17-OHP and below 50 pg/ml for plasma ACTH. The mean observation period after the drug changes was 10 months. Mean concentrations of serum 17-OHP decreased from 48.6 ng/ml to 10.1 ng/ml, as did those of plasma ACTH from 198.0 pg/ml to 35.1 pg/ml. The average drug requirement for CA was 33.9 mg/m^2, while it was 20.3 mg/ m^2 for HC when disease control was stable. The relationship can be expressed as an equation, HC = 0.58 × CA; the coefficient was substantially lower than the conventionally reported dose ratio of 0.8. The authors concluded that CA is inferior to HC as the substitution therapy in patients with CAH.

Inada H, Imamura T, Nakajima R, Yamano T. Poor response to substitution therapy with cortisone acetate in patients with congenital adrenal hyperplasia. *Clin Pediatr Endocrinol.* 2004;13:11–15.

Editor's Comment: *CA may be used as the glucocorticoid component of substitution therapy for CAH. However, the paper by Inada and a previous paper by Jinno[1] indicate that oral administration of CA was inappropriate as glucocorticoid replacement therapy in patients with 21-OHD. The Jinno group compared the effect of CA with that of HC in 7 neonates with 21-OHD. From the time of diagnosis, CA was administered to 4 subjects, while HC was given to the other 3 subjects. The serum cortisol (F), cortisone (E), and 17-OHP in these 7 neonates with 21-OHD were compared with 118 normal subjects. In the normal subjects, serum E concentrations were greater than F during the first 2 months after birth, whereas F concentrations exceeded E after 2 months of age (Table). Infants with 21-OHD who received high CA doses had extremely low serum F concentrations, while 17-OHP concentrations were high until about 2 months of age. Thereafter, the serum F exceeded E, and 17-OHP became fully suppressed even though infants received moderate doses of CA. In contrast, HC administration successfully normalized serum 17-OHP in the neonatal period. With temporary switching from HC to CA, serum F concentrations immediately decreased and 17-OHP concentrations increased. Thus, conversion of E to F may be limited during early infancy, adversely affecting the treatment with CA. Jinno and colleagues also noted that CA was inappropriate as a glucocorticoid replacement during early infancy in patients with 21-OHD.*

To this author's knowledge, no comparative studies of CA and HC treatment during the neonatal period or infancy have been published. In the Jinno et al study, serum E concentration exceeded that of F in normal subjects until the age of 2 months. Conversion of E to F by CA may be difficult, as production of E is greater than that of F in the adrenal cortex from the fetal period to approximately 2 months of age. The predominant E production may reflect age-related morphologic findings of the neonatal adrenal. The human fetus extensively converted F to E (an oxidation reaction), but was unable to convert E to F (a reduction reaction).

At term, in normal infants, each adrenal gland weighs 4 to 5 g, more than 80% of which consists of an inner, hyperemic fetal zone. In this zone, conversion of F to E overshadows conversion of E to F. One-half of the adrenal weight is lost by 1 month of age, and by the age of 1 year the average gland weighs only about 1 g. This postnatal involution of the adrenal cortex involves gradual remodeling of fetal zone cells into the zona fasciculate during the first weeks and months of life. As the fetal zone is associated with a predominance of E, its involution was associated with the age-related changes of serum concentrations of E and F shown in normal subjects.[1]

Activity of 11β-HSD is high in human tissues, especially the inner fetal zone of the adrenal cortex. Results suggest the occurrence of a physiologic inability to respond to treatment with CA during early infancy in patients with 21-OHD, because oxidation by 11β-HSD predominates in the residual fetal cortex.[1] In contrast to cortisone, HC possesses an 11β-hydroxyl group and does not require activation by the enzyme 11β-HSD.

HC should be the drug of choice for substitution therapy in children with CAH. The Japanese Society of Pediatric Endocrinology recommends HC for the maintenance therapy of CAH.

Yoshikazu Nishi, MD

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Growth on Stimulant Medication

Stimulant medication for the treatment of attention deficit hyperactivity disorder (ADHD) has long been suspected to have an adverse effect on linear growth. The first studies concerning this were published in the 1970s and since that time, there have been numerous other studies, which rather than clarifying this relationship, seem to have added to the controversy. Poulton reviewed 29 cohort studies published through September 2004 of children treated with methylphenidate or dexamphetamine. Twenty-two of the studies involved children, 6 involved adolescents or adults close to their adult height, and 1 study included both children and adults. Of the 29 studies, 9 gave results consistent with reduction in height growth while on stimulants and 12 had negative findings. There was a slight difference in the median medication dose (31.4 mg vs 23.9 mg) in those studies which showed significant growth attenuation. Various methods were used to describe height, but the most frequently used

method was height deficit, meaning that the child was a certain amount shorter than he would have been had he continued to grow at a previous rate. Some studies used height z-scores.

The most sensitive studies were the longitudinal studies analyzing periodic observations taken before and after the initial period of treatment. Half of these studies (8 of 16) showed an attenuation of growth on the stimulants by at least 1 method, most reliably a change in height z-scores. The most scientifically rigorous study was one in which children with ADHD were randomly assigned to different treatment groups. This study showed a height deficit of 0.9 cm/yr in the first 14 months and 1.04 cm/yr from 14–24 months in children who received pharmacological treatment. Eight of the longitudinal studies used normative data as the controls, three of which showed an attenuation of height.

The studies of late adolescent and adult heights were mostly cross-sectional, and none showed any significant difference between those treated and the controls. The author stated that many of the studies were of poor quality. However, those of better quality demonstrated a significant association between treatment and attenuated height growth. The conclusion was that despite the large number of studies, most of those failed to detect any adverse affect on growth due to stimulant medication. Many did not stand up to any rigorous analysis. They further stated that it is reasonable to anticipate a reduction in height velocity when children are placed on stimulant medication, but that further studies should be performed in order to better understand the significance of this reduction.

Poulton A. Growth on stimulant medication; clarifying the confusion: a review. *Arch Dis Child*. 2005;90:801–806.

First Editor's Comment: *This paper is a welcome analysis of a large number of studies involving stimulant medications and the measurement of height in children with ADHD. Pediatric endocrinologists are often faced with the question of whether or not stimulant medication will adversely affect growth, and it is very difficult to reference opinions with well-conducted longitudinal trials. Thus, one is left with the conclusion that the results are uncertain. Poulton has shown that at least in those studies that were more rigorously performed, there did seem to be a significant height deficit in these children. However, he also points out that children often do not remain on stimulant medications for the duration of their linear growth. Thus, an overall effect on final height is difficult to discern. This review will hopefully encourage investigators to perform the kinds of studies needed to answer this question conclusively. Such studies need to be randomized, control trials with varying doses of stimulant medications. With so many children currently receiving these medications, such a trial seems feasible.*

William L. Clarke, MD

Second Editor's Comment: *The efficacy of ADHD treatment and the growth of patients was also studied by the MTA Cooperative Group at the National Institutes of Mental Health*[1] *and reviewed in GGH.*[2] *It was clearly documented that there were behavioral benefits in treating ADHD patients, but there was decreased growth (–1.9 cm in height suppression in 24 months). As well, there were weight changes (– 2.5 kg in the first 14 months and –1.22 kg in the following 20 months of treatment). These changes were more prominent in patients who adhered to the medication regimen. However, there were many who stopped taking the medication and thus, the effects were less marked. Suboptimal nutrition may play a role in the reduced growth and weight gain due to the effects of these medications. Thus, when these medications cannot be interrupted, physicians should attempt to overcome the decreased dietary intake and correct any nutrient deficits.*

Fima Lifshitz, MD

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2. *Growth Genet Horm*. 2004;20:62.

Cardiovascular Effects of Adolescent Growth Hormone Deficiency

The metabolic effects of growth hormone (GH) led to FDA-approval of rhGH therapy for GH deficiency (GHD) in adults, even though they have no prospect of height benefits. These effects include improvements in body composition, serum lipid levels, and cardiac function, among others. Lanes and colleagues sought to determine whether cardiovascular function is already altered in adolescents with GHD. These authors compared 10 adolescents with GHD on GH treatment (0.03 mg/kg/d for a mean of 3.8 ± 1.1 yr), 12 adolescents with untreated GHD (4 of whom had previously received 1.6 ± 0.2 yr of treatment but had been off GH treatment for 3.4 ± 1.2 yr due to financial reasons) and 14 healthy adolescent controls. The 3 groups were similar in chronologic age, bone age, height (but not height z-score), BMI, pubertal distribution (65-70% Tanner stages 2-4; remainder prepubertal), blood pressure, and pulse. GHD was defined by abnormally low serum IGF-I and IGFBP-3 concentrations plus failure on clonidine/L-DOPA stimulation testing (peak GH concentrations were 3.2 ± 2.4 and 3.0 ± 2.3 µg/L with a range of 0.9–5.6 µg/L).

A pediatric cardiologist and his technician, blinded to the GH status of the adolescents, performed echocardiography, carotid sonography, and measurement of endothelium-dependent vasodilation. For this last measurement, Doppler ultrasonography was used to quantify right brachial artery blood flow and brachial artery diameter before and 45 to 60 seconds after release of 5 minutes of 300 mm Hg applied by a standard sphygmomanometer cuff to the forearm (to induce hyperemia). They also measured, during echocardiography, the epicardial adipose tissue on the right ventricle, which was described in 2003 as a correlate with MRI measurement of abdominal visceral fat, clinical parameters of metabolic syndrome, and hence, cardiovascular risk in adults.[1]

Left ventricular mass was significantly lower in the untreated and treated GHD groups than the normal controls, although left ventricular posterior wall and interventricular septal thicknesses were both similar across groups. Left ventricular ejection fraction (%) was also similar, but the controls had significantly larger end systolic and end diastolic volumes than the 2 GHD groups. Carotid artery intima-media thickness did not differ, but the hyperemia-induced increases in brachial artery diameter and blood flow were both related to GH status; vasodilation was lower in the untreated GHD group than in the treated and control groups, and blood flow was greatest in the treated GHD group. Epicardial adipose tissue, which correlated positively with BMI in all 3 groups, was significantly greater in the untreated GHD adolescents than the other groups. Thus, GHD has been associated with decreased cardiac size, increased large-artery stiffness (IGF-I has a direct releasing effect on nitric oxide, an endothelial relaxing factor), and increased epicardial adipose tissue (a correlate of cardiovascular risk factors in adults).

Lanes R, Soros A, Flores K, Gunczler P, Carrillo E, Bandel J. Endothelial function, carotid artery intima-media thickness, epicardial adipose tissue, and left ventricular mass and function in growth hormone-deficient adolescents: Apparent effects of growth hormone treatment on these parameters. *J Clin Endocrinol Metab*. 2005;90:3978–3982.

Editor's Comment: *Quite extensive data have been accumulating on the cardiovascular effects of GH and GHD. I refer the reader to references 2 and 3 for reviews of GH effects and reference 4 for review of IGF-I effects on cardiovascular system. Growth hormone replacement therapy for GHD in adults is too new to allow analysis of the ultimate question; that is, if rhGH can significantly ameliorate the increased cardiovascular mortality seen in adults with GHD. The interim markers are encouraging; however, most of the work has examined adults.[5] Lanes and colleagues alert us that potentially detrimental cardiovascular changes can be seen in patients with GHD as early as adolescence. Thus, cardiovascular health joins body composition issues (muscle mass and bone mineralization) as factors to consider in optimizing GH treatment during the transition period, the time between cessation of linear growth and attainment of full adult body maturity.[6]*

Adda Grimberg, MD

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